

**UNIVERSIDADE FEDERAL DE SÃO CARLOS**  
**CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE**  
**PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA E RECURSOS NATURAIS**

**VINÍCIUS DE LIMA DANTAS**

**O PAPEL DO FOGO NA ESTRUTURAÇÃO FUNCIONAL E FILOGENÉTICA DE  
SAVANAS E FLORESTAS TROPICAIS**

**Orientador:** Marco Antônio P. L. Batalha

**SÃO CARLOS**

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Tese apresentada ao Programa de Pós-Graduação em Ecologia e Recursos Naturais, do Centro de Ciências Biológicas e da Saúde da Universidade Federal de São Carlos como parte dos requisitos para a obtenção do título de doutor em Ciências com ênfase em Ecologia e Recursos Naturais.

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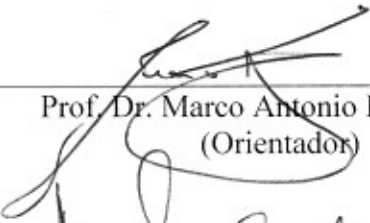
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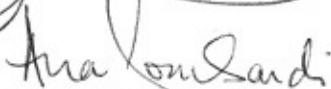
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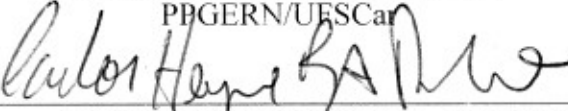
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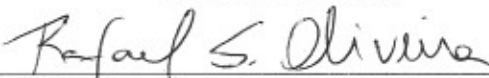
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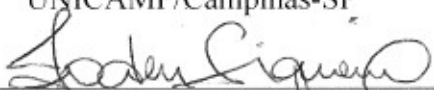
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RESUMO – As savanas ocupam aproximadamente um sexto da superfície terrestre. A origem desse bioma no final do Mioceno parece estar intimamente associada a um aumento nas frequências de incêndio, resultando na expansão de savanas e campos sobre áreas anteriormente dominadas por florestas. O fogo também tem sido apontado como um fator central regulando a distribuição atual de savanas e florestas nos trópicos. Assim, é impossível entender a dinâmica temporal desses biomas sem compreender os mecanismos através dos quais o fogo interage com o ambiente e com os atributos funcionais da vegetação para moldar a assembleia de comunidades. Nesse sentido, a presente tese focou-se em três questões relacionados à ecologia do fogo em savanas e florestas tropicais: (1) Qual é o papel do fogo na estruturação dos atributos funcionais e na assembleia de comunidades em savanas? (2) Qual é o papel do fogo na distribuição dos biomas de savana e floresta tropical nos trópicos úmidos? (3) Quais são os principais preditores da assembleia de comunidades em savanas filtradas pelo fogo e por quais mecanismos operam? Para responder a essas perguntas nós coletamos informações sobre atributos funcionais medidos em plantas individuais e variáveis ambientais no Parque Nacional das Emas (Goiás, Brasil) e utilizamos informações sobre histórico de fogo, obtidos através de sensoriamento remoto. Essas informações foram utilizadas para testar modelos específicos sobre a dinâmica da vegetação nesses sistemas. Nossos resultados nos permitiram concluir que: (1) As savanas tropicais úmidas são comunidades filtradas pelo fogo, de forma que variações nas frequências de incêndios dentro das amplitudes que atualmente ocorrem nelas não explicam uma grande parte da diversidade funcional; (2) Nos trópicos úmidos, as savanas e florestas são dois estados vegetacionais estáveis mantidos por mecanismos de retroalimentação entre o fogo e a vegetação. (3) Em savanas heterogêneas filtradas pelo fogo, a distribuição de recursos como água e nutrientes é o principal fator regulando a assembleia da comunidade.

ABSTRACT – The savanna is a major biome covering one sixth of the Earth land surface. Its origin in the late Miocene seems to be tightly related to an increase in fire frequencies, promoting the spread of savannas and grasslands over areas previously dominated by forest vegetation. Fire has also been pointed out as an important factor regulating the current distribution of tropical savanna and forest biomes. Thus, it is impossible to fully understand the temporal dynamics of these biomes without knowledge on the mechanisms by which fire interacts with the environment and with plant traits to shape community assembly in the tropics. Thus, we focused in three broad questions related to the fire ecology of savanna and forest biomes: (1) What is the role of fire in structuring trait variability in savannas? (2) What is the role of fire in regulating the distribution of tropical savanna and forest biomes? (3) What are the main predictors of community assembly in fire filtered savannas and by which mechanisms they operate? To answer these questions we collected field data on plant traits measured at the individual level, soil and topographic information in Emas National Park (central Brazil) and obtained fire history information from remote sensing data. We used such data to test specific models about the dynamics of the vegetation in savanna and forest biomes. Our studies allowed us to conclude that: (1) Mesic savannas are fire-filtered in the sense that the variations in fire frequency currently observed in these savannas do not explain large fractions of the functional diversity and of the community assembly. (2) In the wet tropics, savannas and forests are two coexisting stable states with contrasting functional traits and assembly processes, regulated by fire-plant feedbacks. (3) In patchy fire-filtered savannas, community assembly is shaped by water and nutrient availabilities.

## I – INTRODUÇÃO GERAL

O bioma savana ocupa aproximadamente um sexto da superfície terrestre (Grace et al. 2006). Estudos paleontológicos sugerem que o bioma se originou e adquiriu sua atual extensão há aproximadamente 10 milhões de anos atrás, coincidindo com a expansão e dominância das gramíneas  $C_4$  em escala global (Cerling et al. 1997, Beerling & Osborn 2006). Essa ascensão foi inicialmente atribuída a uma diminuição na concentração de gás carbônico atmosférico que teria favorecido gramíneas  $C_4$  (gramíneas dotadas de mecanismos capazes de concentrar gás carbônico nos espaços intracelulares; Sage 2004) em detrimento das gramíneas  $C_3$  (Cerling et al. 1997, Ehleringer et al. 1997).

À época em que foi proposto, esse modelo tinha apenas apoio indireto, já que ainda não era possível estimar as concentrações de gás carbônico atmosférico dos paleoclimas com precisão. Assim, as principais bases para o modelo eram duas. Em primeiro lugar, experimentos de competição entre gramíneas  $C_3$  e  $C_4$ , sob várias condições de concentração de gás carbônico e temperatura, apontavam para a superioridade competitiva de gramíneas  $C_4$  em relação a  $C_3$  sob as condições prevalentes nos trópicos (Ehleringer et al. 1997). Em segundo lugar, uma diminuição na temperatura global no final do Mioceno, inferida por meio de isótopos estáveis de oxigênio, indicaria uma diminuição no efeito estufa (Raymo & Ruddiman 1992, Zachos et al. 2001), possivelmente resultante de uma diminuição no gás carbônico atmosférico (Cerling et al. 1997).

Com o surgimento de novas tecnologias isotópicas se tornou possível estimar diretamente as concentrações de gás carbônico na atmosfera do Mioceno. Evidências de diversas fontes independentes falharam em detectar qualquer variação importante nas concentrações de gás carbônico que coincidissem com a ascensão das gramíneas  $C_4$  (DePonto & Pollard 2003, Beerling & Osborn 2006). Além disso, evidências fósseis sugerem que, antes da dominância por gramíneas  $C_4$ , a paisagem não era majoritariamente dominada por gramíneas  $C_3$ , mas por

árvores C<sub>3</sub>, formando bosques de estrutura variáveis (Keeley & Rundell 2005). Uma vez que as gramíneas C<sub>4</sub> são intolerantes à sombra, um modelo baseado puramente em competição não poderia explicar como gramíneas C<sub>4</sub> substituíram florestas de plantas C<sub>3</sub>. Assim, apesar da evolução das gramíneas C<sub>4</sub>, cerca de 25 milhões de anos antes, coincidir com uma queda global na concentração de gás carbônico atmosférico (Sage 2004, Christin et al. 2008), outros mecanismos seriam necessários para explicar a expansão das savanas e campos no final do Mioceno.

Em contrapartida, evidências fósseis apontam para o surgimento de um clima fortemente estacional no período, caracterizado por invernos secos e verões quentes e chuvosos nos trópicos (Keeley & Rundell 2005). Além disso, outras evidências revelaram um grande aumento no fluxo de partículas de carvão para o fundo dos oceanos no mesmo período, indicando um forte aumento na frequência de incêndios (Berling & Osborn 2006). Essas evidências, combinadas com estudos recentes sobre o papel atual do fogo nas savanas e seus ciclos anuais nos trópicos (Higgins et al. 2000, Bond et al. 2005, Sankaran et al. 2005), intimamente ligados aos ciclos das gramíneas C<sub>4</sub>, indicavam que o fogo teria sido um fator chave no surgimento do bioma savana.

Um estudo filogenético posterior incluindo espécies da América do Sul (Simon et al. 2009) forneceria um apoio adicional a essa hipótese. Esse estudo apresenta evidências de que o surgimento das atuais espécies lenhosas de plantas das savanas sul-americanas coincide com o final do Mioceno. Uma vez que as principais características diferenciando as espécies de savana de suas irmãs de florestas secas e úmidas são suas altas resistências e resiliências ao fogo, as linhagens de cerrado parecem ter evoluído *in situ* em resposta aos novos regimes de incêndio que emergiram no período (Simon et al. 2009). Considerando a grande importância que fogo parece ter na origem do bioma savana, pode-se concluir que é impossível entender

completamente a dinâmica das savanas sem compreender o seu papel enquanto fator ecológico, atuando na seleção de linhagens de plantas e moldando as características estruturais e funcionais das comunidades tropicais.

Atualmente, diversas regiões do planeta, sobretudo nos trópicos, possuem um clima úmido o suficiente para suportar a ocorrência de florestas, mas atualmente são cobertas por vegetações abertas de savana ou campo (Bond et al. 2005, Sankaran et al. 2005, Bond 2008). Essas diferenças têm sido atribuídas principalmente ao papel do fogo nessas regiões, mas há um consenso de que os efeitos do fogo são complexos e não lineares, interagindo com diversas outras variáveis (Bond et al. 2005, Staver et al. 2011, Murphy & Bowman 2012). Atualmente, sabe-se que ao gerar ciclos consecutivos de mortalidade aérea de árvores jovens seguidas de rebrotamento basal, o fogo mantém as árvores em um tamanho reduzido em savanas, impedindo que cresçam, se adensem e formem um dossel fechado (Kellman 1984; Higgins et al. 2000; Hoffmann et al. 2009). Assim, o fogo exerce gargalos demográficos nas populações plantas, levando a uma alta dominância de indivíduos anões, e esses gargalos exercem fortes pressões seletivas em favor de plantas resistentes ao fogo (Bond & van Wilgen 1996, Bond 2008). Dentro das populações, apenas uma pequena parcela dos indivíduos, aqueles de crescimento mais rápido (em espessura da casca ou total), seriam capazes de tornarem-se resistentes ao fogo antes do incêndio seguinte (Wakeling et al. 2011, Lawes et al. 2013). Entretanto, são os regimes de fogo e não a ocorrência de fogo que determinam qual estratégia é mais eficiente para escapar do fogo em diferentes savanas (Dantas & Pausas 2013).

Com base nesse processo, modelos baseados em estados estáveis e em mecanismos de retroalimentação entre o fogo e a vegetação têm sido propostos para explicar a distribuição de savanas e florestas nos trópicos (Kellman 1984, Higgins et al. 2000, Bond 2008, Hoffmann et

al. 2012). O mais recente deles propõe que a transição de uma vegetação de savana para uma de florestas depende de dois limiares ecológicos (Hoffmann et al. 2012). O primeiro seria em nível individual e estaria relacionado ao momento crítico em que uma dada planta desenvolve uma casca grossa o suficiente para se tornar independente do fogo (Hoffmann et al. 2012). O segundo seria em nível de comunidade, quando uma comunidade de árvores desenvolve um dossel suficientemente denso e alto para reduzir a flamabilidade do sistema, tanto pela supressão de gramíneas  $C_4$ , como pela alteração das condições microclimáticas (Hoffmann et al. 2012, Murphy and Bowman 2012). A probabilidade de atingir esses limiares dependeria do tamanho do intervalo entre dois incêndios e da disponibilidade dos recursos necessários para sustentar um crescimento rápido durante esse intervalo. Além disso, essa transição dependeria dos atributos funcionais das espécies presentes na área, como seus investimentos em cascas grossas, das densidades das folhas em suas copas e de suas taxas de crescimento intrínsecas (Hoffmann et al. 2012, Murphy and Bowman 2012). Enquanto as espécies que evoluíram em savana possuem cascas especialmente grossas que lhes conferem uma alta resistência ao fogo (Hoffmann et al. 2009), espécies de floresta podem levar décadas para formar essas cascas (Hoffmann et al. 2009). Por outro lado, espécies de floresta têm altas taxas de crescimento e copas densas, o que lhes permitiria fechar o dossel da comunidade rapidamente (Hoffmann et al. 2012). Dessa forma, a probabilidade de cruzar o primeiro limiar seria maior para espécies de savana enquanto que a probabilidade de cruzar o segundo seria maior para espécies de floresta (Hoffmann et al. 2012). Apesar da coerência desse modelo, estudos empíricos apresentando evidências que o apoiem ainda são escassos.

Assim, a presente tese focou-se em três temas relacionados à ecologia do fogo em savanas e florestas tropicais: (1) Qual é o papel do fogo na estruturação funcional de comunidades vegetais de savana? (2) Por quais mecanismos o fogo estrutura a distribuição dos biomas de savana e floresta tropical nos trópicos úmidos? (3) Quais são os principais preditores da

assembleia de comunidades em savanas filtradas pelo fogo e por quais mecanismos eles operam?

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## II – CAPÍTULO 1: O PAPEL DO FOGO NA ESTRUTURAÇÃO DE TRAÇOS FUNCIONAIS EM SAVANAS NEOTROPICAIS\*

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# **The role of fire in structuring trait variability in Neotropical savannas**

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## **Abstract**

Intraspecific trait variability plays a fundamental role in community structure and dynamics; however, few studies have evaluated its relative importance to the overall response of communities to environmental pressures. Since fire is considered a key factor in Neotropical savannas, we investigated in what extent the functional effects of fire in a Brazilian savanna occurs via intra- or inter- specific trait variability. We sampled 12 traits in communities subjected to three fire regimes in the last 12 years: annual, biennial, and protected. To evaluate fire's relative effects we fitted a general linear mixed models with species as random and fire as fixed factors, using: 1) all species in the communities (i.e., considering intra and interspecific variabilities); 2) 18 species common to all fire regimes (i.e., intraspecific variability only), and; 3) all species with their overall average trait values (i.e., interspecific variability only). We assessed the relative role of inter- and intra- specific variability by comparing the significance of each trait in the three analyses. We also compared the within and between fire variabilities with a variance component analysis. Five traits presented larger intraspecific than interspecific variability, and the main effect of fire occurred at the intraspecific level. These results support that it is important to consider intraspecific variability to fully understand fire-prone communities. Moreover, trait variability was larger within than among fire regimes. Thus, fire may act more as an external filter, preventing some of the species from the regional pool to colonize the cerrado, than as an internal factor structuring the already filtered cerrado communities.

**Keywords:** cerrado; fire regimes; intraspecific variability; plant traits; variance components.

## **Introduction**

Most studies on the effects of biotic interactions and abiotic drivers in plant communities have focused on interspecific trait variability, considering plant traits as species average values (for example, Westoby et al. 2002; Wright et al. 2005; Pausas and Verdú 2008; Kraft and Ackerly 2010). Including intraspecific variability in community studies is a promising path to improve our ability for understanding assembly processes, habitat selection, and community functioning, regardless of whether this variability has a genetic basis or not (McGill et al. 2006; Albert et al. 2010; Bolnick et al. 2011). Intraspecific variability is associated with greater resource use efficiency, greater microsite occupancy, and higher resilience at community level (Bolnick et al. 2011). Moreover, since intraspecific variability plays a fundamental role in species coexistence and interactions (Violle et al. 2012), the evaluation of the phenotypic community structure is significantly affected by its inclusion (Jung et al. 2010; Cianciaruso et al. *in press*). Few studies have evaluated the relative importance of intraspecific variability to the response of communities to environmental gradients (Albert et al. 2010). However, the recent rise of variance-based statistics have provided a common ground for comparing and decomposing the effects of community processes into its intra- and inter-specific components (de Bello et al. 2010; Messier et al. 2010; Violle et al. 2012). Plant communities are generally assembled by two main processes: habitat filtering and limiting similarity (Weiher and Keddy 1995; Wilson et al. 1999). Since, habitat filtering tends to reduce interspecific trait variability (Kraft et al. 2008; Pausas and Verdú 2010), in strongly filtered communities the intraspecific variability is expected to play an important relative role in community structure and resilience.

Fire exerts an important pressure in many biomes worldwide (Pausas and Keeley 2009), acting as an environmental filter in plant communities (Verdú and Pausas 2007) and as a driving force for species evolution (Keeley et al. 2011, 2012; He et al 2012). Most studies on

fire effects in plant traits at a community scale have focused on changes related to shifts in species composition (e.g., Pausas et al. 2004; Saura-Mas and Lloret 2007; Verdú and Pausas 2007; Pausas and Verdú 2008; Moretti and Legg 2009). However, recent studies emphasised the role of intraspecific trait variability in fire-prone ecosystems (Moreira et al. 2012; Pausas et al. 2012; Cianciaruso et al. *in press*). Nevertheless, to what extent the effects of recurrent fire in plant communities are related to intraspecific variability remains poorly explored.

The origin of the Neotropical savannas is associated with the spread of flammable C<sub>4</sub> grasses in South America about 4-8 million years ago (Berling & Osborn 2006; Simon et al. 2009). The appearance of such grasses increased the frequency of fires, imposing a strong filtering, as well as a selective pressure on the coexisting woody species. The resulting communities have an herbaceous layer of tall flammable grasses coexisting with woody species adapted to survive fires. Typical wildfires in the Brazilian savannas (known as “cerrado”) occur in the wet season (generating small fires) or during the transition from dry to wet (generating larger fires; Miranda et al. 2009). In this vegetation type, because grasses are often tall and trees relatively short, there is no clear fuel gap between grasses and woody plant, and fires often affect both the herbaceous layer and the coexisting shrubs and trees, generating regimes of frequent low intensity fires (crown and surface fires). Consequently, the long fire history has filtered cerrado communities by preventing the establishment of species not adapted to fire, and by driving the evolution of a fire-adapted flora (Simon et al. 2009), thus limiting interspecific phenotypic and functional variability.

As long as in strongly filtered communities interspecific trait variability is reduced, intraspecific variability should have an important relative role in community response to disturbances. We predict that in cerrado, which has been strongly filtered by fire, intraspecific trait variability plays a fundamental role in the functional response to different fire regime, and should account for an important fraction of the overall variability of the community. If so,

the relative importance of changes in species composition observed in cerrado communities submitted to different fire regimes (Silva and Batalha 2008) should not explain most of the functional trait variability; instead, an important part of the variability should occur at the intraspecific level. To evaluate this, we studied 12 functional traits of woody plants occurring in different fire regimes in a cerrado reserve. The current management of cerrado reserves generated a patchwork of fire regimes, from annual fires to fire-protected zones, suitable for studying trait variability generated by fire.

## **Material and methods**

### *Study area*

We used data collected at Emas National Park (ENP; central Brazil: 17°49'-18°28'S and 52°39'-53°10'W), a World Natural Heritage site (Unesco 2001) located in the Brazilian Central Plateau. ENP is one of the most important cerrado reserves in Brazil (Conservation International 1999). The reserve has a tropical warm wet climate, with dry winters (*Aw* type following Köppen 1931); most soils are Oxisols. The topography is mostly flat and homogeneous. The cerrado includes a wide range of physiognomies, from open grassland to woodland with different tree densities (Coutinho 1990); most of these physiognomies occur inside ENP, but open formations are the most abundant ones.

The ENP was burned annually by farmers in the dry season until 1984 to promote forage regrowth. In 1984, the reserve was fenced, cattle were removed, and a fire exclusion policy was established (Ramos-Neto and Pivello 2000). During the fire exclusion period (1984-1994), fires burned large areas every three years, due to dry biomass accumulation (França et al. 2007). Since 1994, annual and biennial prescribed burns within firebreaks have been applied in 10 km<sup>2</sup> strips to remove plant dry mass and prevent large fire (França et al. 2007). These management actions have driven the average fire return interval to about 6-7 years



(França et al. 2007), but the outcome of this management is a patchwork of different fire regimes within the park.

### *Sampling*

Data were collected during a three month period of the 2006 rainy season, from areas with similar physiognomies (open savannas) and topography (flat areas), but different fire history during the last 12 years (Silva and Batalha 2010): (1) an annually burned firebreak ( $18^{\circ}18'50''\text{S}$ ,  $52^{\circ}54'00''\text{W}$ ; sampled one year after the last fire); (2) an area burned 5 times during the last 12 year, with a mean fire interval of 1.8 ( $18^{\circ}19'01''\text{S}$ ,  $52^{\circ}54'10''\text{W}$ ; sampled 3 years since the last fire); and (3) a site protected from fire for 12 years ( $18^{\circ}17'28''\text{S}$ ,  $52^{\circ}53'41''\text{W}$ ). For simplicity, we refer to these fire regimes as ‘annual’, ‘biennial’ and ‘protected’. In each fire regime, 250 points separated by 10-m intervals were sampled along a 2.5 km transect. At each point, four plants were sampled using the point-center-quarter method (Müller-Dombois and Ellenberg 1974). Thus, in each of the fire regimes, a total of 1,000 individual plants were sampled, from 50 woody species. The distance between adjacent transects ranged from 0.5 to 2 km, and since the sites were all located in flat and homogeneous areas, no important environmental variability was expected among transects.

For each species, we randomly picked seven individuals per fire regime by sampling their identification code from the pool containing the code of all individuals of the target species. When seven individuals were not available, we made an extra effort within the fire regime to complete the dataset. In these seven selected individuals per species and fire regime, we measured the following 12 functional attributes: plant height, basal area, bark thickness, wood density, specific leaf area, leaf dry matter content, and the concentration of nitrogen, phosphorous, potassium, calcium, magnesium, and sulphur in leaves. All traits were measured according to Cornelissen et al. (2003), except for chemical leaf traits, which were measured

following Allen (1989). Wood density and chemical leaf attributes were measured in five individuals per species and fire regime. For multi-stemmed plants, basal area, and bark thickness were measured at the main (oldest) stem that was not killed by fire. To measure woody density we collected a woody cylindrical sample of the plant's main stem (or as close as possible to it), removed all the bark, and, using a calliper, we measured its diameter and length. We used these measures to estimate the volume of the sample. The woody density was obtained by dividing the dry mass of the sample (oven-dried at 80°C for 72 h) by its fresh volume. Bark thicknesses were estimated by removing a bark sample from the main stem with a knife and measuring its thickness with a calliper.

Leaf traits were measured in completely expanded, hardened, and mature sun leaves, with no symptoms of pathogen, herbivory, or senescence, and haphazardly selected from the outer canopy. To measure specific leaf area, we collected two leaves from each sampled individual, kept them in a cooler and subsequently scanned them. We used this digitalized images to estimate leaf area with the Image J 1.33 software (Rasband 2004). We determined specific leaf area dividing the area of the fresh leaves by their oven-dried (80°C for 72 h) mass.

### *Analyses*

To evaluate the role of fire in trait variability, and the relative importance of intraspecific and interspecific variability, we performed three analyses: firstly, we fitted a general linear mixed model (GLMM) with fire regimes (protected, biennial, annual) as fixed factor and species as random factor using the actual trait values of all individuals in each fire regime (that is, considering intraspecific and interspecific variabilities). We used an equal number of individuals per species in each fire regime (seven) so that the effects of variability observed were not affected by differences in abundance, but exclusively by the inter and intra-specific variabilities. Secondly, to assess whether this variability was mainly related to changes in

species composition or to intraspecific trait variability, we excluded intraspecific variability by calculating an overall mean trait values for each species, regardless of the fire regime, and matched these mean trait values with species presence and absence per fire regime. We performed an analysis of variance with fire as fixed factor, which permitted to assess trait variability explained by differences in species composition only. Finally, to test whether fire generated intraspecific variability, we fitted a GLMM using the 18 species common to all fire regimes (that is, excluding interspecific variability), using fire as fixed factor and species as random factor. Out of the 50 species sampled, these 18 species (Supplementary material - Table A1) represented: 28% of the woody species occurring across the three sites, 46% of the woody species in the protected (unburned) site, 50% of the woody species in the biennially burned site and 64% of the woody species in the annually burned site (Silva and Batalha 2010). We assessed the relative role of inter- and intra- specific variability generated by fire, by comparing the significance of each trait in the three analyses. We repeated all the analyses considering two fire regimes in order to perform the three pairwise comparison tests for each trait.

We also compared the variability generated by fire with the overall trait variability to assess the importance of fire in generating trait variability by performing a variance component analysis in a GLMM including all species and fire regimes, with fire regimes nested in species as a random factor. We performed the same analysis using only the 18 common species to partition the variability in intraspecific and interspecific variability and investigated whether the variability within species was larger than the variability among species, as a measure of the importance of the intraspecific variability to the overall trait variability. When necessary, we log-transformed the trait values prior to the analysis to approximate normality. All analyses were carried out with the “nlme” and “ape” libraries in the R environment (R Development Core Team 2009).

## **Results**

Fire regimes significantly affected most traits when both, species composition and intraspecific variability were considered (Table 1 and 2). Removing the intraspecific variability resulted in the loss of most of the significant relationships, and only basal area and plant height (out of 12 traits) were significantly related to fire regimes. In contrast, removing the variability in species composition (when only the 18 species common to all fire regimes were considered) did not alter most of the relationship between fire regimes and traits observed in the whole community approach (Table 1 and 3). In both the first and the last case, plant height was lowest in the annually burned site and highest in the protected site (Table 2 and 3). Specific leaf area was lowest at the annually burned site in comparison with the protected site. Leaf nitrogen, magnesium, and phosphorus were lowest and leaf calcium was highest in the annually burned site. For any trait fire was the main factor explaining trait variability, since the within fire variability was larger than the between fire variability (Table 2). Nevertheless, the proportion of the variability explained by fire increases when the effect of species composition is excluded (Table 3).

## **Discussion**

Fire regimes were significantly related to trait variability at landscape scale in cerrado. Although the Brazilian cerrado has been subject to recurrent fires during the last few millions years (Simon et al. 2009), current fire regimes still have a significant impact in generating trait variability (Silva and Batalha et al. 2010; Cianciaruso et al. *in press*), and especially intraspecific trait variability. In fact, removing all intraspecific variability resulted in the loss of most of the significant relationships, whereas removing the variability in species composition did not, even though the species composition was significantly different among

fire regimes (see Silva and Batalha 2008). Thus, the intraspecific variability seems to represent an important fraction of the overall variability generated by fire and cannot be neglected in future studies in cerrado. These results, combined with others reporting the importance of fire in generating intraspecific variability (for instance, Moreira et al. 2012; Pausas et al. 2012), suggest that studies on the effects of fire in plant communities would benefit from a more detailed observation of trait variability beyond species average values. All these results support our hypothesis of the prominent importance of intraspecific variability in strongly fire-filtered communities.

Partitioning the overall trait variability of the 18 species common to all fire regimes resulted in that all but five traits presented a larger intra- than inter- specific variability (Table 3). The combined effect of fire in filtering the species of the regional pool for thousands of years, reducing the overall interspecific variability, and recent fire regimes in generating intraspecific variability may result in this relatively large importance of intraspecific variability. To what extent this pattern may also occur in other strongly filtered communities worldwide remains to be explored.

Plants burned annually were shorter and had higher basal areas than plants protected from fire. This is likely to be a consequence of the recurrent topkilling by fire under annual fire frequencies, that is, fire interfering with plant development, causing a “bonsai effect” (or Gulliver syndrome; Higgins et al. 2007). Thus, these results support that fire acts opening savanna physiognomies. Also, bark was thinner under annual fires. Although frequent surface fires in savanna environments lead to an expectation of greater investment in bark (Keeley and Zedler 1998; Jackson et al. 1999; He et al. 2012), annual crown-fires may have partially destroyed the bark of adults and limited the building of a thick protective bark. This suggests that the bark thickness observed in cerrado plants may be more than enough for protecting them from frequent, but low intensity, cerrado fires. Also, we found plants in the protected

area to have higher specific leaf area than plants in the frequently burned sites; more frequent burning promoted smaller, thicker and tougher leaves. Since all the sites studied consisted of open physiognomies, it is unlikely that the differences in leaf trait result of different light incidence among fire regimes. Specific leaf area is considered a key trait that summarise the allocation trade-off between growth and defence or reserves (Chapin et al. 1993; Gurvich et al. 200). Therefore, lower specific leaf area values observed in the annually burned plants may be a consequence of slower growth rates, due to a higher allocation to reserves for annual resprouting (Moreira et al. *in press*). Also, they may represent a great investment in leaf anti-herbivory resistance to protect the leaves from the increment in herbivory that usually takes place after fires (Lopes and Vasconcelos 2011; Kersch-Becker and Lewinsohn 2012).

The fact that frequently burned plants had the lowest foliar nutrients (N, P, Mg), thicker and tougher leaves (lower specific leaf areas) and higher Ca concentration, as opposed to the protected plants, suggest the existence of a nutrient stress (Coley et al. 1985; Ward et al. 1997; McLaughlin and Wimmer 1999; Craine 2009). In the cerrado, there is evidence of fires occurring with less than 3 year interval, resulting in nutrient loss from system (Pivello and Coutinho 1992). Since cerrado soils are nutrient-poor (Ruggiero et al. 2002; Dantas and Batalha 2011), individuals under high fire frequency could be facing a significant nutrient limitation. Although this reduction in the nutrient availability may be the consequence of the direct biomass consumption by fire, nutrient stress may also be a consequence of the indirect effects of increased herbivory after fire (Lopes and Vasconcelos 2011; Kersch-Becker and Lewinsohn 2012). Leaf traits are believed to be labile and present high plasticity (see Hoffmann and Franco 2003 for an example in the cerrado). However, the chemical leaf traits presented a low proportion of its variability explained by intraspecific variability in comparison with other traits. Recent studies reported chemical leaf traits to be more evolutionary conserved than other leaf traits (Cianciaruso et al. *in press*) suggesting an

association between phylogenetic conservatism and intraspecific variability, which could be a subject to further investigation.

When partitioning the variance into its intra- and interspecific components, we observed that the variability within fire was greater than among fire regimes. Thus, although fire exerted a significant effect in trait variability at the intraspecific level, there was still a large variability not explained by fire regimes, suggesting that although fire acts as an important external filter (*sensu* Violle et al. 2012) in cerrado preventing plants from the regional pool, without the appropriated traits, from colonising the cerrado (Simon et al. 2009), other internal factors may be responsible for most of the trait variability observed at the landscape. Nevertheless, previous studies also found larger within (local) than among communities (regional) intraspecific variability (see Supplementary material in Albert et al. 2010; Moreira et al. 2012), suggesting that this could be a general pattern in many plant communities worldwide.

In recent years, investigating the role of intraspecific variability has been regarded as a fundamental issue to community ecology, allowing a more precise prediction of community responses to environmental changes, and a greater understanding of community functioning (Albert et al. 2010, Messier et al 2010, Boldnick et al. 2011, Violle et al. 2012). Indeed, there is increasing evidence suggesting that intraspecific variability may be as large as or even larger than interspecific variability (Bello et al. 2010; Messier et al. 2010; our results). Thus, to understand fully the effects of fire in cerrado, and possibly in other strongly filtered communities, intraspecific trait variability need to be considered.

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Table 1. Results of the GLMM for the effects of fire regime (protected, biennial and annual) on 12 functional traits considering 1) Inter + intraspecific variability, which combines all species in the community and individual plant traits (see Table 2; dfl=2, df2 ranges between 374 and 593); 2) interspecific variability only (dfl=2, df2 ranges between 96 and 98); and 3) Intraspecific variability among the 18 species most common species (see Table 3; dfl=2, df2 ranges between 246 and 352). Shown in parentheses is the subset of the data used in each analysis: number of species; trait resolution.

Trait	Inter + intraspecific (all; individual values)		Interspecific (all; mean species values)		Intraspecific (18 common species; individual values)	
	F	P	F	P	F	P
Basal area (cm <sup>2</sup> )	0.704	0.495	3.88	<b>0.024</b>	4.206	<b>0.041</b>
Plant height (cm)	32.934	<b>&lt;0.001</b>	6.625	<b>0.002</b>	86.541	<b>&lt;0.001</b>
Wood density (mg · mm <sup>-3</sup> )	2.088	0.1252	2.105	0.127	0.968	0.326
Bark thickness (mm)	33.661	<b>&lt;0.001</b>	2.439	0.093	49.479	<b>&lt;0.001</b>
Specific leaf area (mm <sup>2</sup> · mg <sup>-1</sup> )	7.891	<b>&lt;0.001</b>	0.225	0.799	8.694	<b>0.003</b>
Leaf dry matter content (mg · g <sup>-1</sup> )	0.696	0.499	0.312	0.733	0.991	0.320
Leaf nitrogen (mg · g <sup>-1</sup> )	4.531	<b>0.0113</b>	0.477	0.622	34.670	<b>&lt;0.001</b>
Leaf phosphorous (mg · g <sup>-1</sup> )	14.936	<b>&lt;0.001</b>	0.187	0.83	58.185	<b>&lt;0.001</b>
Leaf calcium (mg · g <sup>-1</sup> )	9.389	<b>&lt;0.001</b>	0.440	0.646	16.158	<b>&lt;0.001</b>
Leaf magnesium (mg · g <sup>-1</sup> )	12.604	<b>&lt;0.001</b>	0.585	0.559	3.350	0.068
Leaf potassium (mg · g <sup>-1</sup> )	2.487	0.0843	0.163	0.850	0.006	0.937
Leaf sulphur (mg · g <sup>-1</sup> )	0.701	0.4967	0.501	0.608	0.674	0.412

Table 2. Trait values of all species within the three fire regimes and results of variance component analysis comparing fire type and species. “Protected” are sites with 12 years of the fire exclusion; “Biennial” and “Annual” refers to fire frequency during this period. Trait values are means  $\pm$  SD (see text for sample size). In each row, values with different letters indicates significant differences in trait values among pairs of fire regimes determined by a general linear mixed model with species as random and fire as fixed factors

Plant traits	Fire regime			Variability		
	Protected	Biennial	Annual	Among fires	Within fire	Among species
Basal area (cm <sup>2</sup> )	51.47 <sup>a</sup> $\pm$ 66.67	53.68 <sup>a</sup> $\pm$ 87.11	40.10 <sup>a</sup> $\pm$ 42.33	0.00	0.76	0.24
Plant height (m)	1.78 <sup>a</sup> $\pm$ 0.96	1.49 <sup>b</sup> $\pm$ 1.00	0.78 <sup>c</sup> $\pm$ 0.58	0.08	0.46	0.45
Bark thickness (mm)	6.72 <sup>a</sup> $\pm$ 4.67	5.33 <sup>b</sup> $\pm$ 3.77	3.13 <sup>c</sup> $\pm$ 3.61	0.19	0.56	0.25
Wood density (mg $\cdot$ mm <sup>-3</sup> )	0.44 <sup>a</sup> $\pm$ 0.23	0.37 <sup>a</sup> $\pm$ 0.11	0.44 <sup>a</sup> $\pm$ 0.21	0.12	0.56	0.32
Specific leaf area (mm <sup>2</sup> $\cdot$ mg <sup>-1</sup> )	5.99 <sup>a</sup> $\pm$ 2.31	5.93 <sup>a</sup> $\pm$ 1.70	5.74 <sup>b</sup> $\pm$ 1.41	0.09	0.45	0.46
Leaf dry matter content (mg $\cdot$ g <sup>-1</sup> )	468.92 <sup>a</sup> $\pm$ 141.80	462.26 <sup>a</sup> $\pm$ 134.93	459.82 <sup>a</sup> $\pm$ 80.62	0.04	0.68	0.28
Leaf nitrogen (mg $\cdot$ g <sup>-1</sup> )	16.26 $\pm$ 5.86 <sup>a</sup>	15.01 $\pm$ 4.65 <sup>b</sup>	16.37 $\pm$ 4.54 <sup>a</sup>	0.16	0.21	0.63
Leaf phosphorous (mg $\cdot$ g <sup>-1</sup> )	0.87 <sup>a</sup> $\pm$ 0.24	0.89 <sup>b</sup> $\pm$ 0.18	0.93 <sup>c</sup> $\pm$ 0.19	0.17	0.31	0.53
Leaf potassium (mg $\cdot$ g <sup>-1</sup> )	4.70 <sup>a</sup> $\pm$ 3.06	5.26 <sup>a</sup> $\pm$ 2.89	4.85 <sup>a</sup> $\pm$ 2.40	0.05	0.33	0.62
Leaf calcium (mg $\cdot$ g <sup>-1</sup> )	3.14 <sup>a</sup> $\pm$ 1.95	3.08 <sup>a</sup> $\pm$ 1.73	3.09 <sup>b</sup> $\pm$ 1.67	0.10	0.31	0.59
Leaf magnesium (mg $\cdot$ g <sup>-1</sup> )	1.24 <sup>a</sup> $\pm$ 0.78	1.35 <sup>b</sup> $\pm$ 0.78	1.54 <sup>b</sup> $\pm$ 1.00	0.12	0.36	0.52
Leaf sulphur (mg $\cdot$ g <sup>-1</sup> )	0.70 <sup>a</sup> $\pm$ 0.18	0.67 <sup>a</sup> $\pm$ 0.16	0.67 <sup>a</sup> $\pm$ 0.18	0.20	0.52	0.28



Table 3. Trait values of the 18 species common in the three fire regimes. Values are mean ( $\pm$  SD) of each trait in fire regime, and their variance components (scaled; among and within three fire regimes and among species). “Protected” represents 12 years of fire exclusion; “Biennial” and “Annual” refers to fire frequency during a 12 years period. In each row, values with different letters represent significant differences in trait values among fire regimes determined by a general linear mixed model with species as random and fire as fixed factors

Plant traits	Fire regime			Intraspecific		
	Protected	Biennial	Annual	Among	Within	Among
				fires	fires	species
Basal area (cm <sup>2</sup> )	37.43 <sup>a</sup> $\pm$ 45.60	35.44 <sup>a</sup> $\pm$ 33.71	49.85 <sup>b</sup> $\pm$ 68.26	0.055	0.679	0.266
Plant height (m)	1.42 <sup>a</sup> $\pm$ 0.66	1.20 <sup>b</sup> $\pm$ 0.75	0.76 <sup>c</sup> $\pm$ 0.56	0.385	0.503	0.112
Bark thickness (mm)	6.30 <sup>a</sup> $\pm$ 4.24	5.06 <sup>b</sup> $\pm$ 4.54	5.08 <sup>c</sup> $\pm$ 4.77	0.287	0.479	0.234
Wood density (mg $\cdot$ mm <sup>-3</sup> )	0.39 <sup>a</sup> $\pm$ 0.11	0.38 <sup>a</sup> $\pm$ 0.12	0.40 <sup>a</sup> $\pm$ 0.17	0.006	0.553	0.442
Specific leaf area (mm <sup>2</sup> $\cdot$ mg <sup>-1</sup> )	6.21 <sup>a</sup> $\pm$ 1.85	6.05 <sup>ab</sup> $\pm$ 1.61	5.75 <sup>b</sup> $\pm$ 1.23	0.078	0.424	0.498
Leaf dry matter content (mg $\cdot$ g <sup>-1</sup> )	440.36 <sup>a</sup> $\pm$ 83.39	477.75 <sup>b</sup> $\pm$ 171.89	453.62 <sup>ab</sup> $\pm$ 73.07	0.068	0.573	0.360
Leaf nitrogen (mg $\cdot$ g <sup>-1</sup> )	16.44 <sup>a</sup> $\pm$ 5.35	15.11 <sup>b</sup> $\pm$ 5.33	14.23 <sup>c</sup> $\pm$ 5.21	0.100	0.209	0.681
Leaf phosphorous (mg $\cdot$ g <sup>-1</sup> )	0.91 <sup>a</sup> $\pm$ 0.18	0.85 <sup>b</sup> $\pm$ 0.18	0.78 <sup>c</sup> $\pm$ 0.17	0.235	0.335	0.431
Leaf potassium (mg $\cdot$ g <sup>-1</sup> )	2.86 <sup>a</sup> $\pm$ 1.95	2.74 <sup>a</sup> $\pm$ 1.74	2.45 <sup>b</sup> $\pm$ 2.00	0.053	0.337	0.611
Leaf calcium (mg $\cdot$ g <sup>-1</sup> )	1.40 <sup>b</sup> $\pm$ 1.18	1.13 <sup>a</sup> $\pm$ 0.59	1.21 <sup>a</sup> $\pm$ 0.73	0.074	0.261	0.666
Leaf magnesium (mg $\cdot$ g <sup>-1</sup> )	4.68 <sup>a</sup> $\pm$ 2.25	4.90 <sup>a</sup> $\pm$ 3.20	4.63 <sup>a</sup> $\pm$ 3.28	0.104	0.317	0.579
Leaf sulphur (mg $\cdot$ g <sup>-1</sup> )	0.69 <sup>a</sup> $\pm$ 0.16	0.72 <sup>a</sup> $\pm$ 0.17	0.70 <sup>a</sup> $\pm$ 0.18	0.130	0.745	0.125

III – CAPÍTULO 2: O FOGO PROVOCA LIMIARES FUNCIONAIS NA TRANSIÇÃO  
ENTRE SAVANA E FLORESTA\*

\* Artigo publicado em 2013 no periódico *Ecology* com o título “Fire drives functional thresholds on the savana-forest transition”

**Running head:** Fire drives functional thresholds

## **Fire drives functional thresholds on the savanna-forest transition**

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**Abstract.** In tropical landscapes, vegetation patches with contrasting tree densities are distributed as mosaics. However, the locations of patches and densities of trees within them cannot be predicted by climate models alone. It has been proposed that plant-fire feedbacks drive functional thresholds at a landscape scale, thereby maintaining open (savannas) and closed (forest) communities as two distinct stable states. However, there is little rigorous field evidence for this threshold model. Here, we aim to provide support for such a model from a field perspective and to analyze the functional and phylogenetic consequences of fire in a Brazilian savanna landscape (Cerrado). We hypothesize that in tropical landscapes, savanna and forest are two stable states maintained by plant-fire feedbacks. If so, their functional and diversity attributes should change abruptly along a community closure gradient. We set 98 plots along a gradient from open savanna to closed forest in the Brazilian Cerrado and tested for a threshold pattern in nine functional traits, five soil features, and seven diversity indicators. We then tested whether the threshold pattern was associated with different fire regimes. Most community attributes presented a threshold pattern on the savanna-forest transition with coinciding breakpoints. The thresholds separated two community states: (1) open environments with low-diversity communities growing in poor soils and dominated by plants that are highly resistant to high-intensity fires; and (2) closed environments with highly diverse plant communities growing in more fertile soils and dominated by shade-tolerant species that efficiently prevent light from reaching the understory. In addition, each state was associated with contrasting fire regimes. Our results are consistent with the hypothesis that forest and savannas are two coexisting stable states with contrasting patterns of function and diversity that are regulated by fire-plant feedbacks; our results also shed light on the mechanism driving each state. Overall, our results support the idea that fire plays an important role in regulating the distribution of savanna and forest biomes in tropical landscapes.

**Key words:** alternative stable states, Cerrado, community dynamics, ecological thresholds, fire regimes, phylogenetic diversity, physiognomic gradient, plant-fire feedback, plant functional traits, savanna-forest systems, tropical biomes.

## **Introduction**

Forest and savanna are two major terrestrial biomes dominating tropical landscapes (Woodward et al. 2004). Although their occurrences at a global scale may be predicted by climate-based models, at regional to local scales patches of contrasting tree densities may occur within the same climatic conditions (Hoffmann *et al.* 2005, Bond 2008, Hirota et al. 2011, Staver *et al.* 2011, Ratnan *et al.* 2011, Murphy and Bowman 2012). Consequently, more complex models have been proposed to explain the differences between climate-based predictions and the actual vegetation cover in tropical landscapes (Bond *et al.* 2005, Archibald *et al.* 2009, Lehmann *et al.* 2011, Pueyo *et al.* 2010, Staver and Levin 2012). There is an emerging consensus that fire plays a fundamental role in these systems and that its relationship with vegetation dynamics is more complex than what a simple correlative model would predict (Bond and Keeley 2005, Staver *et al.* 2011, Murphy and Bowman 2012).

A model based on alternative stable state theory and tree growth-fire interaction (the ‘tree growth-fire interaction’ model) has been recently proposed to explain the dynamics of savanna-forest systems (Hoffmann *et al.* 2012a, Murphy and Bowman 2012). Such a model was built upon the assumption that savanna and forest are two alternative stable states with distinct structures and functions that are maintained by positive plant-fire stabilizing feedbacks. The transition from savanna to forest would be dependent on the crossing of two ecological thresholds: (1) the *fire-resistance threshold*, i.e., the stage at which an individual tree achieves a critical bark thickness and becomes fire-resistant, surpassing the fire-

vulnerable juvenile stage (Hoffmann *et al.* 2009); and (2) the *fire-suppression threshold*, i.e., the stage at which a woodland achieves sufficient canopy closure to dramatically reduce the flammability of the system (for example, decreased light and C<sub>4</sub> grass density and increased moisture; Hoffmann *et al.* 2012a). Because species common in forest environments need more time to achieve critical bark thickness than species typical of savanna, the crossing of these thresholds would be dependent upon sufficient fire intervals (Hoffmann *et al.* 2012a). In landscapes where fire frequency is high, the occurrence of forest is predicted to be associated to plant growth enhancers (for example, high water or nutrient availability; Hoffmann *et al.* 2009, Murphy and Bowman 2012). Although this idea is straightforward, most of the evidence for stable states in forest-savanna systems comes from indirect inferences and theoretical models (Hennenberg *et al.* 2006, Hoffmann *et al.* 2012a, Staver and Levin 2012). Because fire inhibition may be achieved at tree densities much lower than those observed in forests (Trauernicht *et al.* 2012), finding field evidence of these critical switching points and their consequences at the community scale would provide a more mechanistic understanding of the processes driving stable states in the tropics.

Within this framework we aim to find evidence of functional and diversity shifts consistent with two vegetation states in the Brazilian Cerrado domain, a fire-prone mosaic of savannas and forests with variable structures (Oliveira-Filho and Ratter 2002, Hoffmann *et al.* 2005). In this system annual rainfall and dry season soil water availability have lower predictive power for plant physiognomies than in Australian and African savanna-forest systems (Murphy and Bowman 2012), and except for the driest sites all levels of tree cover may occur within the same climatic conditions (Goodland and Pollard 1973, Oliveira-Filho and Ratter 2002, Hoffmann *et al.* 2005). Although early studies attributed the physiognomic gradient of the Cerrado to a gradient of fertility (Goodland and Polard 1973), increasing

evidence suggests that soil fertility and texture alone are weak predictors of tree density (Lehmann *et al.* 2011, Staver *et al.* 2011, Hoffmann *et al.* 2012a) because: (1) savanna and forests have been found to be associated with a diversity of edaphic conditions (Haridasan 1992, Hoffmann *et al.* 2012a); (2) forest species are able to colonize and grow in these savannas in the absence of fire (Hoffmann and Franco 2003, Hoffmann *et al.* 2009); and (3) there is sufficient nutrient stock in the soils of open Cerrado physiognomies to support a forest biomass (Bond 2010). Forests within Cerrado landscapes tend to experience more fires than forests in other savanna-forest systems (Murphy and Bowman 2012), but the fire frequency is much lower than in open Cerrado physiognomies (Hoffmann *et al.* 2009, 2012a). Because Cerrado landscapes include patches with significant variability in tree densities, they offer an excellent opportunity to study functional changes and threshold effects along this community closure gradient. We hypothesized that in Cerrado landscapes, despite the existence of a gradient of community structure (from open savannas to closed forests), there are two well-defined stable states of community function, each associated with contrasting levels of community closure and maintained by different fire regimes. Consequently, we predicted the existence of an abrupt shift in community parameters along a community closure gradient reflecting distinct vegetation states, particularly in the community parameters related to fire-plant feedbacks. Given that open communities are more flammable and that fire opens up the communities (Gambiza *et al.* 2000, Hennenberg *et al.* 2006, Higgins *et al.* 2007), we expected fire feedbacks to enhance not only the compositional and phylogenetic differences between the alternative states but also the differences in fire escape strategies in a fire-prone landscape. Specifically, we expect fire-plant feedbacks to segregate open communities dominated by fire-resistant species with early investments in bark thickness and high vegetative recovery rates from communities dominated by fire-sensitive species, which

protect themselves from fire by efficiently closing the canopy and reducing the flammability of the system (Hoffmann *et al.* 2012b). In landscapes where fire is frequent forests are predicted to be restricted to high resource soils, which decreases the time needed to accumulate fire-protective barks and to achieve canopy closure and thus fire inhibition (Hoffmann *et al.* 2009). Thus, we expect soil fertility to change abruptly along the community closure gradient. Finally, the consequences of different ecological settings (light and nutrient availability, fire regime) between open and closed communities should also have implications for species assembly and community diversity. To evaluate these predictions, we sampled plant functional traits, soil nutrients, and plant diversity attributes along a savanna-forest gradient (that is, a community closure gradient) in a Cerrado reserve (Emas National Park, Brazil) and tested for the existence of consistent thresholds separating communities with contrasting fire regimes. Our results will be consistent with two vegetation states maintained by plant-fire stabilizing feedbacks if (1) community attributes (particularly the ones discussed above) show a significant breakpoint along the community closure gradient; (2) the threshold model defined by this breakpoint explains substantially more variability than a linear model; and (3) there are different fire regimes on the different sides of the threshold.

## **Materials and Methods**

### *Study site*

Emas National Park (ENP) is a 132,000 ha World Heritage site containing flora, fauna, and key habitats that characterize the Cerrado. It is located in the Brazilian Central Plateau, in the southwest of the state of Goiás (17°49'-18°28'S and 52°39'-53°10'W). All of the neotropical savanna physiognomies can be found within the park, but open Cerrado physiognomies predominate, covering approximately 68% of the area; dense savannas and



forests cover approximately 25% and 1.2% of the area, respectively (Ramos-Neto and Pivello 2000; see Appendix A). The climate is tropical humid (Aw, following Köppen 1931), with two well-defined seasons: a dry season (from May to September) and a warmer rainy season (from October to April). The annual rainfall varies between 1200 and 2000 mm, concentrated mainly from October to March, and mean annual temperature is 24.6°C (Ramos-Neto and Pivello 2000). The soils are mainly dystrophic Oxisols. Until 1984, the park was exploited by cattle ranchers, and fire was used to generate fodder (Ramos-Neto and Pivello 2000). After a large fire in 1994 that burned most of the park, the fire management policy of the reserve was changed: natural fires are now allowed in the park, and a network of annually burned firebreaks limits the spread of fires (Ramos-Neto and Pivello 2000). Hence, large fires burning throughout the park became less frequent. During the sampling period, the ENP was a mosaic of patches with differing fire histories.

### *Sampling*

Using a map of time since last fire and a GIS, we set 100 5 m x 5 m plots stratified by fire age and located near small roads. For practical and legal reasons, plots that fell in an inaccessible region of the park were discarded. We also discarded plots with fewer than three woody individuals (stem diameter at the ground level  $\geq 3$  cm) and those with fewer than two species. Because the areas that were unburnt for more than 15 years were all relatively small forest fragments, most of which were inaccessible, we distributed 20 plots systematically in two accessible forest fragments. The final analysis included 98 plots with different fire ages (from 1 to more than 32 years) and different vegetation structures. Forest plots corresponded to typical semi-deciduous forests of the region and were set every 50 meters along the trail that crosses each of the forest fragments and at 20 m from the trail. The ENP is mostly flat

and there were no strong topographic differences among the sampled plots. During the rainy seasons of 2009/10 and 2010/11 we sampled, in each plot, all woody individuals with stem diameter at the ground level  $\geq 3$  cm and identified them to species. For each individual we measured plant height, stem diameter, bark thickness and leaf toughness, and collected leaf, wood and soil samples for further analyses (see “*Field sampling*” in Appendix B). In the laboratory, we calculated the height-to-diameter ratio and bark thickness-to-diameter ratio and measured the specific leaf area, leaf nitrogen, phosphorus and potassium content, and wood density (see “*Plant functional traits*” in Appendix B). These functional traits are related to fire resistance (bark thickness-to-diameter ratio; Lawes *et al.* 2012), vegetative recovery ratios (wood density; Curran *et al.* 2008), topkill (height-to-diameter ratio; Dantas *et al.* 2012), shade-tolerance (specific leaf area, Westoby *et al.* 2002) and nutrient use (specific leaf area, leaf toughness and leaf nutrient contents; Westoby *et al.* 2002, Craine 2009). Traits were measured in all individual plants because intraspecific variability is an important component of trait variability that influences species assembly (Jung *et al.* 2010, Dantas *et al.* 2013, Violle *et al.* 2012). Composite soil samples from each plot were sent to the Soil Science Laboratory at the University of São Paulo, where soil organic matter content (OM) and the concentration of available phosphorus (P), total nitrogen (N), cations (Ca, Mg, K) and aluminum (Al) were determined (see “*Soil data*” in Appendix B).

### *Fire history*

We used annual satellite images from 1979 to 2010 to determine fire years in each plot. Images from 1979-1983 were obtained from the satellites Landsat 1, 2, and 3 (MSS; 80 m resolution), whereas images from 1984-2010 were obtained from the satellites Landsat 5 and 7 (TM and ETM; 30 m resolution). The mean fire interval for each plot was determined as the

inverse of fire-frequency in the plot. We assigned the maximum fire interval (32 years) for plots that had not burned within the period we examined.

### *Community closure index*

To test whether community parameters displayed a threshold relationship along the savanna-to-forest gradient, we calculated a community closure index (CCI) as the sum of the woody volume of all plants in the plot, standardized to vary from 0 to 1. We computed the woody volume of each individual tree assuming a cone shape (i.e., basal area x height / 3). The index was used as an indicator of the light environment, from open communities (CCI close to 0) to closed communities (CCI close to 1). We did not consider tree cover because of the low resolution at the extremes of the gradient. Basal area was also discarded because plots with the same basal area (especially at intermediate basal area values) may vary substantially in structure depending on the height of the plants. Our index is expected to be more sensitive to fine-scale variability in light incidence than tree cover or basal area, and is therefore more appropriate for testing our hypothesis (see Appendix C). Indeed, evidence points out that woody volume is better in distinguishing Cerrado physiognomies than basal area (Batalha *et al.* 2001). Moreover, combined plant height and stem diameters are good predictors of canopy leaf biomass in the Cerrado (Delitti *et al.* 2006), and there is evidence that wood volume is also a good predictor (Salis *et al.* 2006). Thus, we expected CCI to reflect the changes in structure and the light environment along the transition from open savanna to forest. The CCI enabled us to order the plots according to community closure and test for threshold patterns in community parameters along the gradient.

### *Numerical analyses*

We computed alpha and beta diversity for each plot along the CCI gradient, considering both traditional (non-phylogenetic) as well as phylogenetic-based indices, as described in Appendix B.

To test for threshold-type relationships (i.e., abrupt changes) among community attributes along the CCI gradient (against the null hypotheses of no pattern or a simple linear pattern), we used the following procedure for each community attribute: (1) we first tested for significant structural changes in the distribution of the community parameter along the CCI gradient by fitting an ordinary least square model before and after every possible breakpoint in the variable distribution and computing F-statistics for all potential breakpoints. The breakpoint with the greatest F-value for each variable was tested for significance with a supF test for structural change (Hansen 1997); (2) we then fit a linear regression to test for linear relationships between the community parameter and CCI; and finally (3) we fit a piecewise regression to variables that presented significant breakpoints using the breakpoint values obtained in step 1. Piecewise regressions are models in which two or more lines are fit to the data and joined by breakpoints representing thresholds (Toms and Lesperance 2003). We fit a simple piecewise regression model with one breakpoint and two linear regressions fit at each side of the threshold value of CCI obtained in step 1. When necessary, we log-transformed the data to improve normality of the residuals. A threshold relationship between CCI and the parameter was inferred when (1) there was a significant breakpoint (supF test, above); and (2) the piecewise model fit significantly better and had lower AICc than the linear model. Finding a significant breakpoint but not a better fit of the piecewise model compared to the linear model would suggest that there is a functional change between the two vegetation states but that the switch is not so abrupt. We used the mean of all threshold values of CCI obtained in

the structural change analysis to separate the area into two states (low and high CCI). We then compared mean fire intervals between the two states with Student's t-test. We carried out all the analyses in R v 2.11 (R Development Team 2011) using the “betapart”, “picante”, “strucchange” and “vegan” libraries.

## **Results**

We sampled a total of 710 individuals of 98 species in 39 taxonomic families. All of the soil variables and diversity indices and all but one of the functional traits presented a significant breakpoint (supF test; Table 1). Most parameters with a significant breakpoint also showed an improved fit when using the piecewise model compared to a linear model (Table 1; Fig. 1; Appendix D), suggesting that the breakpoint is rather abrupt. Soil features presented threshold patterns with breakpoints between 0.56 and 0.58 CCI that separated areas with low soil fertility (at low CCI) from areas with high soil fertility (at high CCI). All but three functional traits (leaf nutrient contents) presented a threshold pattern with breakpoints between 0.56 and 0.59 CCI (Table 1), although the functional change in leaf toughness was relatively small. Breakpoints separated communities dominated by plants with low height-to-diameter and high bark thickness-to-diameter ratios, low wood density, and tougher leaves with low specific areas (at low CCI) from communities with the opposite characteristics (at high CCI). Most of the beta-diversity indicators (phylogenetic and non-phylogenetic) presented a clear threshold-type distribution in relation to CCI, suggesting a compositional and phylogenetic turnover. However, alpha diversity measures (mean phylogenetic distance, Shannon diversity, and species richness) and abundance-weighted composition dissimilarity presented significant breakpoints with a non-significant or slightly significant increased fit by the piecewise model (Table 1), suggesting that the shifts in these parameters are not abrupt.

Considering all significant breakpoints in the supF test, the mean threshold was located at CCI = 0.57 (SD = 0.009). This mean threshold separated two clear states with contrasting community attributes (Fig. 2, Appendix E). The mean fire interval differed significantly before and after this mean threshold (t-test;  $t = -9.085$ ,  $p < 0.001$ ) with clearly longer mean fire intervals associated with plots located at the high end of the CCI gradient (Fig. 3).

## Discussion

Soil properties, phylogenetic and non-phylogenetic beta-diversities, and most of the plant functional traits presented a threshold pattern along the CCI gradient with coinciding breakpoints – providing strong evidence of a functional threshold along the forest-savanna gradient. Open environments consisted of communities growing on poor soil and dominated by short species with early investments in thick barks, low wood density and with thick and tough leaves (high toughness and low specific area). In contrast, closed communities grow in more fertile soils and include plants having the opposite functional attributes. Moreover, we found contrasting fire regimes on the two sides of the threshold, with open formations showing shorter fire intervals than closed formations and a switch from communities dominated by fire-resistant plants to communities dominated by shade tolerant species that compensate for their lack of fire resistance by efficiently closing the canopy (i.e., reducing flammability). Overall, our results are consistent with the theoretical model of fire-plant feedbacks as main drivers of the coexistence of two stable states, savanna and forest (Hoffmann *et al.* 2012a, Murphy and Bowman 2012). In this context, we provide the first field-based evidence for a community-level threshold separating two vegetation states with distinct functional and phylogenetic characteristics and associated with different fire regimes.

The functional characteristics of plants associated with the two states support the idea that these communities are maintained by plant-fire feedbacks. In open communities, grasses are often tall, whereas trees and shrubs are small, leaving no fuel gaps between grasses and woody plants (Appendix A-f). Consequently, fires in open communities affect the whole plant (Appendix A-a). However, Cerrado plants typically have thick, often corky barks that protect the whole stem (see Appendix A-d for an example). Depending on the fire intensity, the bark effectively protects a portion of fully scorched branches, and the plant resprouts epicormically from the protected lateral buds. This pattern results in trees with branched architectures. When combined with recurrent topkill and the need for stocking resources for resprouting, it results in slow growth in height (low height-to-diameter ratios; Higgins *et al.* 2007, Dantas *et al.* 2013) and light woods (high rates of vegetative resprout; Curran *et al.* 2008). This pattern differs from other savannas (e.g., many oak savannas and pine savannas) in which plants have a relatively thick stem bark and there is a clear fuel gap between the grass layer and the overstory protecting branches and leaves from surface fires (Glitzenstein *et al.* 1995, Peterson and Reich 2001, Keeley *et al.* 2012). In contrast, open Cerrado trees with corky bark in both stem and branches are similar to some Mediterranean trees with very thick bark that resprout epicormically after crown fires (Pausas 1997, Catry *et al.* 2012). In closed communities, fire is inhibited because shade-tolerant plants quickly occupy light gaps, preventing light-demanding C<sub>4</sub> grasses from establishing. This effect, combined with the changes in microclimatic condition promoted by a closed canopy, drastically reduces the flammability of the system (Hoffmann *et al.* 2012b), allowing these fire-sensitive shade-tolerant species which possess high wood density to support large crowns (Enquist *et al.* 1999, Markesteijn *et al.* 2011), and fast leaf turnover and high photosynthetic ratios to quickly occupy light gaps (high specific leaf area; Westoby *et al.* 2002). Although bark thickness is the most important trait

preventing tree and stem mortality in neotropical forests and savannas (Hoffmann *et al.* 2009, Brando *et al.* 2012), high wood density, large stem diameter, and high canopies are also associated with reduced levels of stem mortality in tropical savannas and forests (Archibald and Bond 2003, Brando *et al.* 2012). These characteristics of forest species as well as the high absolute bark thickness provided by large stems may be important to prevent low-intensity surface fires from opening the community.

High and low specific leaf areas were associated with closed and open communities, respectively. Specific leaf area is a key trait related to the leaf economic spectrum, and high specific leaf area is associated with high photosynthetic capacity and fast leaf turnover (Westoby *et al.* 2002). These characteristics are largely linked to shade tolerance and promotion because they confer an efficient response to light patchiness (Westoby *et al.* 2002). These attributes may allow forest shade-tolerant species to quickly reach canopy closure in the absence of fire so that mixed communities of shade-tolerant and shade-intolerant species become unstable and rare (Hoffmann *et al.* 2009, Ratnam *et al.* 2011), leading to a threshold pattern. On the open side of the threshold, soils are nutrient poor and recurrent fires cause frequent nutrient losses by consuming live and dead biomass (Pivello and Coutinho, 1992); the need for a large carbohydrate reserve for resprouting in this system may favor plants with high nutrient-use efficiency and leaves with high investments in leaf structure and low palatability to herbivores to avoid nutrient loss (low specific leaf areas and high leaf toughness; Westoby *et al.* 2002, Craine 2009).

All soil nutrients presented a clear threshold pattern with greater fertility in the soil (high nitrogen, phosphorus, sum of bases, organic matter, and low aluminum saturation) associated with closed communities. Nutrient stock analysis has been used to demonstrate that the size of the nutrient pool in the soil of neotropical savannas is not a limiting factor



preventing the development of a forest biomass (Bond 2010). Indeed, many forest species are able to establish and develop in nutrient-poor soils of adjacent neotropical savannas suggesting that the nutrient pool is not a limiting factor to forest species (Hoffmann *et al.* 2009). Moreover, tropical savannas and forest have been found occurring within both nutrient-rich and nutrient-poor soils (Bond 2008, Bond 2010, Hoffmann *et al.* 2012a) and without sufficiently long fire intervals, forest cannot replace savannas, despite soil fertility (Hoffmann *et al.* 2009, 2012a, Rossatto *et al.* 2009). Instead, increasing evidence suggests that the effect of soil on tree growth rates and how it affects the probability of a given community achieving fire inhibition are more likely to be the pathways by which soil affects biome distribution in the tropics (Bond 2010, Hoffmann *et al.* 2012a, Murphy and Bowman 2012). Because the breakpoints in fire-related traits coincide with those of soil, our results are consistent with the idea of fire as the main driver of the thresholds observed here whereas soil fertility decreases the time needed for crossing the fire-inhibition threshold (Hoffmann *et al.* 2009, Hoffmann *et al.* 2012a).

Flammability in the Cerrado is a function of community closure, and it shifts abruptly once a certain level of community closure is achieved. After this point, fire recurrence and/or intensity are predicted to be critically low (Hoffmann *et al.* 2012b). Our results for three decades of fire history support this prediction. The contrasting fire regimes associated with contrasting patterns in community parameters, including fire-related ones, indicate that plant-fire stabilizing feedbacks are linked to changes in fire regimes. Changes in fire regime associated with community closure were also observed in the Australian savannas, where low-intensity fires or fire inhibition was associated with closed canopy communities and high-intensity fires were associated with open-canopy communities (Trauernicht *et al.* 2012). Open communities are subject to frequent fires that often affect most of the plants, whereas in

closed communities fires are rare and, when they occur, are often very low-intensity surface fires (Trauernicht *et al.* 2012). This pattern is most likely related to the inhibition of grassy fuels, the decrease in wind speed and air temperature, and the increase in fuel moisture achieved after crossing a critical community closure threshold (Hoffmann *et al.* 2012b, Trauernicht *et al.* 2012). Although we might have missed some understory fires in closed communities on the satellite images, these fires are of low intensity, as forests tolerate them without open large gaps that could change vegetation state. Moreover, a change from fires that fully burn the trees to understory surface-fires represents an abrupt change in fire intensity consistent with a fire-inhibition threshold and with the stable state theory. Thus, our data provide additional evidence that after a certain canopy closure is achieved, fires that topkill larger plants are absent for long periods, in contrast with the savannas, where the lack of a fuel gap between grasses and woody plants often results in crown-fires.

Phylogenetic and non-phylogenetic beta diversities shifted along the gradient of CCI, reflecting the compositional and phylogenetic differences among open and closed communities. Despite the existence of congeneric forest and savanna species (Hoffmann and Franco 2003, Simon *et al.* 2009), abrupt changes in abiotic conditions associated with high phylobetadiversity (that is, high phylogenetic turnover as measured by the PCD index) are indications of phylogenetic conservatism of species niches and their associated traits (Graham and Fine 2008). Moreover, high beta and phylobetadiversities are expected when there is a limited dispersal of a species from one environment to another, or when an entire clade is locally missing (Graham and Fine 2008). Given that there was not limited dispersal between savanna and forest sites (i.e., they belong to the same regional species pool), the threshold pattern in the two beta diversity indices indicates that the observed differences are ecological rather than biogeographical and emphasizes the different assembly processes between the two

vegetation states (Graham and Fine 2008). Mean phylogenetic distance also presented a breakpoint in its distribution with lower values in open communities. This result is consistent with findings in Mediterranean ecosystems in which high fire activity drives the co-occurrence of closely related species (Verdú and Pausas 2007). Nevertheless, the shifts were not abrupt compared with other community attributes.

In summary, we provided field-based evidence for the existence of functional and diversity thresholds that are consistent with the hypothesis that savanna and forest are two stable states. Plant height-to-diameter and bark thickness-to-diameter ratios, woody density, and specific leaf area presented the most contrast among plant attributes between the two states and these are key traits predicting plant strategies, community assembly, and plant survival in fire-prone landscapes (Westoby *et al.* 2002, Curran *et al.* 2008, Craine 2009, Hoffmann *et al.* 2009, Brando *et al.* 2012, Lawes *et al.* 2012). The strong association between these traits and communities with different fire regimes provides additional support for the idea that fire-plant feedback processes regulate the dynamics of savanna and forest biomes, maintaining them as two different states with contrasting function, diversity, and assembly processes in tropical landscapes.

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Fig. 1: Height-to-diameter ratio, bark thickness-to-diameter ratio, wood density ( $\text{mg mm}^{-3}$ ), and specific leaf area (SLA;  $\text{mm}^2\text{mg}^{-1}$ ) along the community closure index gradient (CCI, from 0 to 1) as examples of the threshold-type relationships found at Emas National Park in central Brazil. Vertical grey lines represent significant breakpoints ( $p < 0.001$ ; Table 1). For more examples see Appendix D.

Fig. 2: Community attributes (standardized from 0 to 1) at each side of the mean threshold (light gray:  $\text{CCI} < 0.57$ ; dark gray:  $\text{CCI} \geq 0.57$ ) along the CCI gradient (for attributes with significant supF test; Table 1). (a) Soil: organic matter (OM), nitrogen (N), phosphorus (P), sum of bases (SB) and aluminum saturation (Al sat). (b) Traits: height-to-diameter ratio (H:D), bark thickness to diameter ratio (BTh:D), wood density (WD), leaf toughness (LTh) and specific leaf area (SLA). (c) Diversity indices: phylogenetic community distance (PCD), Jaccard's beta-diversity (BJac), abundance-based community dissimilarity (Dis.Ab) and presence-absence-based community dissimilarity (Dis.PA). Thick bars show medians, boxes show lower and upper quartile, whiskers show maximum and minimum values and points show outliers.

Fig. 3: Frequency distribution of plots in relation to mean fire interval (MFI; grouped into one-year interval classes) for plots with low community closure index (light grey bars; 98% of the plots have  $\text{MFI} < 6$  years) and for plots with high community closure index (dark grey bars; 79% of the plots have  $\text{MFI} > 30$  years).

Fig 1:

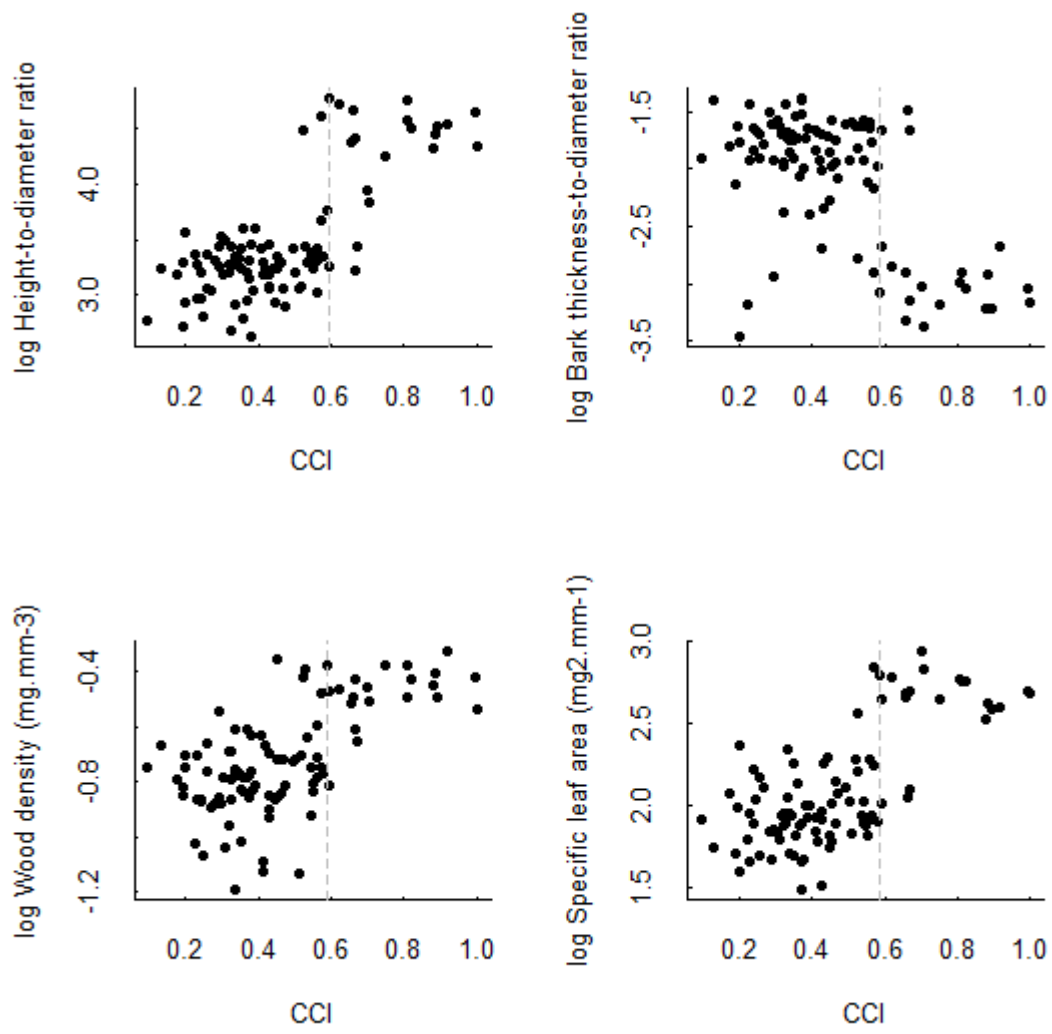


Fig 2:

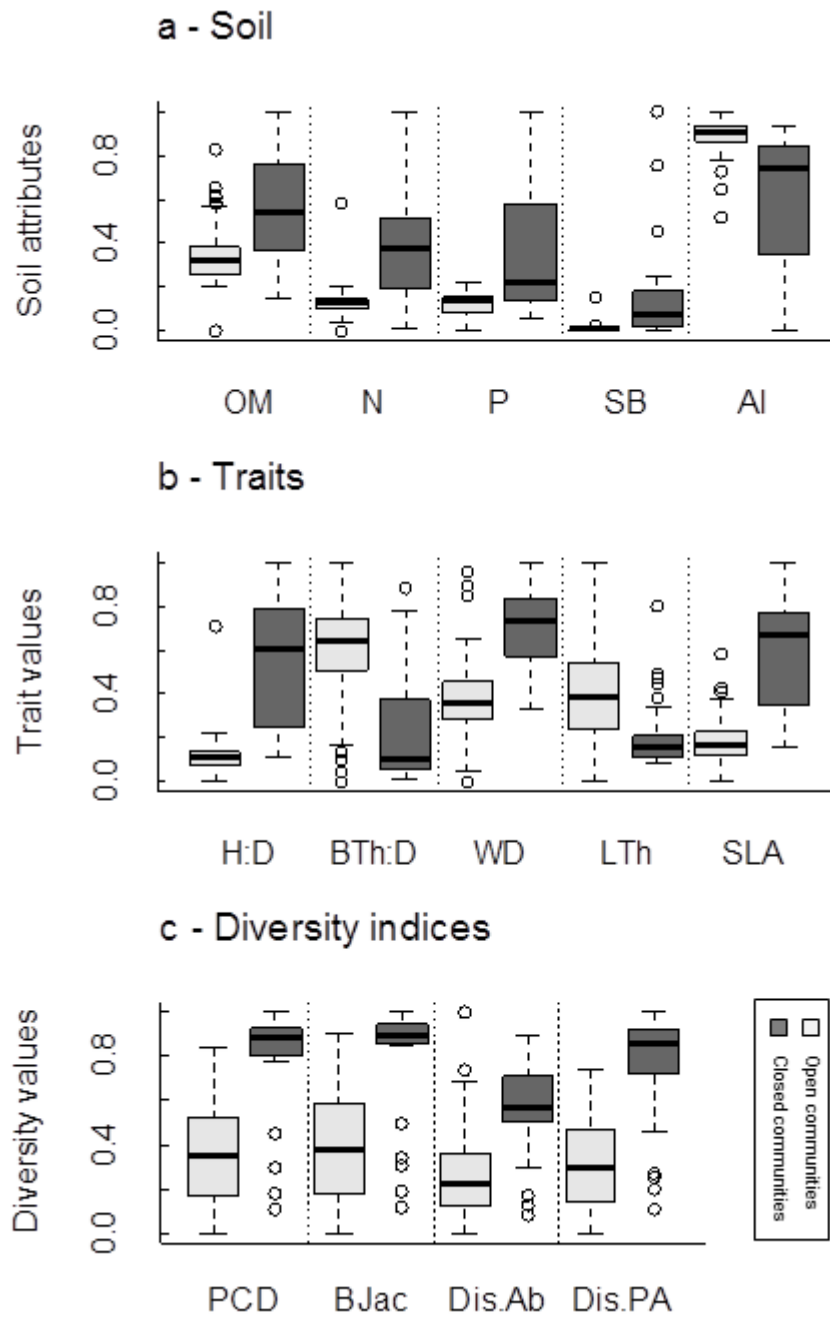
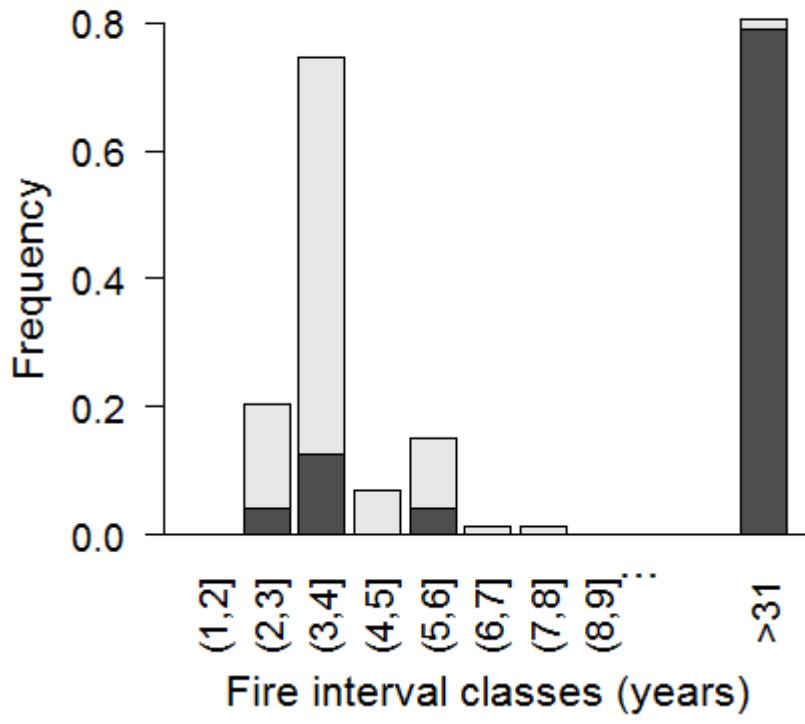


Fig 3:



**Table 1:** Summary of the detection of thresholds in different community attributes (soil properties, plant traits and diversity indices) along the community closure gradient. Structural change analysis (supF test), linear regression and piecewise regression are shown. The comparison between the linear and the piecewise model was performed by an ANOVA (LR vs PR); the changes in AICc are also shown. Thresholds are defined as significant breakpoints (supF test) with a piecewise model explaining significantly more variability than the linear model

Community attributes	supF test		Linear regression (LR)		Piecewise regression (PR)		ANOVA (LR vs PR)		$\Delta$ AICc
	F	Breakpoint	slope	R2	p	R2	P	F	
	Soil organic matter	51.42***	0.57	6.32	0.17	<0.001	0.30	<0.001	
Soil nitrogen	101.62***	0.57	448.15	0.32	<0.001	0.46	<0.001	13.18***	19.82
Soil phosphorus	63.02***	0.57	1.90	0.24	<0.001	0.38	<0.001	10.13***	14.73
Soil sum of basis	36.72***	0.58	4.89	0.12	<0.001	0.35	<0.001	15.98***	24.03
Soil aluminum saturation	74.83***	0.58	-5.11	0.25	<0.001	0.45	<0.001	17.67***	27.05
Height to diameter ratio	172.54***	0.59	98.79	0.52	<0.001	0.64	<0.001	15.03***	52.79
Bark thickness to diameter ratio	61.08***	0.56	-0.02	0.30	<0.001	0.39	<0.001	11.23***	9.04

Wood density	84.56***	0.57	0.04	0.43	<0.001	0.49	<0.001	6.23**	7.79
Leaf toughness	24.94***	0.58	-0.10	0.15	<0.001	0.20	<0.001	3.63*	2.89
Specific leaf area	181.47***	0.57	1.45	0.50	<0.001	0.66	<0.001	21.44***	32.43
Leaf nitrogen	12.43**	0.43	0.04	0.12	<0.001	0.13	0.001	0.79 <sup>ns</sup>	-3.36
Leaf phosphorus	11.24*	0.57	-0.03	0.05	0.032	0.08	0.004	0.18 <sup>ns</sup>	-0.81
Leaf potassium	4.85 <sup>ns</sup>	-	-0.04	0.03	0.078	-	-	-	-
Mean Phylogenetic Distance	39.57***	0.56	12.01	0.26	<0.001	0.30	<0.001	2.38 <sup>ns</sup>	0.46
Phylogenetic Community Distance <sup>a</sup>	75.31***	0.57	11.44	0.30	<0.001	0.44	<0.001	11.84***	17.62
Jacard's beta-diversity <sup>a</sup>	64.59***	0.57	90.87	0.26	<0.001	0.39	<0.001	10.21***	14.86
Composition dissimilarity 1 <sup>a,b</sup>	54.21***	0.57	69.07	0.31	<0.001	0.37	<0.001	4.04*	3.75
Composition dissimilarity 2 <sup>a,c</sup>	99.07***	0.59	39.77	0.39	<0.001	0.50	<0.001	11.21***	16.56
Shannon's diversity	55.22***	0.56	0.18	0.39	<0.001	0.42	<0.001	2.69 <sup>ns</sup>	1.04
Richness	56.07***	0.56	0.84	0.40	<0.001	0.44	<0.001	2.88 <sup>ns</sup>	1.42

a: beta-diversity indices were measured cumulatively along the community closure index gradient; b: abundance-based; c: considering presence/absence only.



\*\*\*  $p < 0.001$ ; \*\*  $p < 0.01$ ; \*  $p < 0.05$ ; <sup>ns</sup> no significance

## **Ecological Archives**

### **Appendix A**

Examples of the variability in community and individual plant attributes in Emas National Park, central Brazil.

### **Appendix B**

Detailed description of field and laboratory methods.

### **Appendix C**

Relationship between the community closure index (CCI) and basal area per hectare.

### **Appendix D**

Soil variables, leaf toughness and diversity indices along the community closure index gradient (CCI) and their breakpoints.

### **Appendix E**

Distribution of basal area and mean height at each side of the threshold.

**Appendix A: Examples of the variability in community and individual plant attributes in Emas National Park, central Brazil.**



a) A woodland cerrado (*cerrado sensu stricto*) six months after a fire, with several top-killed trees and a developed layer of resprouting vegetation; b) one of the sampled closed forests; c) a dense woodland cerrado (*cerrado denso*); d) one example of a typical thick-barked species found in open communities (*Anadenanthera peregrina* (Benth.) Reis, Fabaceae); e) a transitional zone between dense savannas and forests; f) a typical open savanna at the early rainy season, with tall flammable grasses and small trees and shrubs. Photo credits: Vinícius Dantas (a, e), Gabriela Sartori (b), Vivian Kadry (c), Juli Pausas (d), Felipe Noronha (e) and Alessandro Favari (f).

## **Appendix B. Detailed description of the field and laboratory methods**

### *Field sampling*

We estimated bark thickness in the field by measuring with a caliper the depth of penetration of a knife inserted into five haphazardly selected points in the stem at approximately 0.50 m from the ground. We estimated the height of tall trees using a four meter tall aluminum ladder and graduated fiberglass pole; when this was not sufficient, we climbed the trees with a single rope technique and measured the distance from a given point in the canopy to the upper photosynthetic tissue and from this point to the ground using a graduated rope and the above-mentioned fiberglass pole. We measured leaf toughness in the field on five completely expanded and hardened mature leaves, haphazardly selected from the outer canopy, with no sign of herbivory or pathogens (Cornelisen et al. 2003). We measured leaf toughness by drilling each leaf on both sides of the mid-rib with a cone tip of a force gauge penetrometer (Chatillon DFE 010, AMETEK, Berwyn, USA). To collect leaf samples from the canopy of very tall trees, we used a pruner with a fiberglass pole that cut branches of trees up to 11 meters tall; the four meter tall aluminum ladder was also used (Appendix A-b). When those were insufficient, we climbed trees, remove a large branch from the canopy with a pruner or a rope, and collected the material from this sub-sample. In each plot, we collected five topsoil samples (0-5 cm deep; Ruggiero et al. 2002) at each corner of the square plot and at the center.

### *Plant functional traits*

We calculated bark thickness per stem diameter by dividing the mean bark thickness, based on the five field measurements for each individual, by the stem diameter of the tree. We measured wood density from branches collected as close as possible to the main stem following Cornelisen et al. (2003). After removing all the bark of the wood samples, we estimated volume of the wood sample by measuring the diameter and the length of the branch with a caliper and estimating the volume of a cylinder. We obtained wood density by dividing a branch's oven-dried mass (at 80°C for 72 h) by its volume. Wood density is associated with vegetative recovery ratios and mechanical strength (Enquist et al. 1999, Curran et al. 2008). Thus, low woody density is associated with high rates of vegetative recovery whereas high woody density represents high mechanical strength. We estimated leaf toughness per individual by calculating a mean value from the 10 field measurements (see field sampling). Leaf toughness is related to nutrient strategy and resistance to herbivory (Craine 2009). We estimated specific leaf area in five leaves per individual on completely expanded and hardened mature leaves, haphazardly selected from the outer canopy (Cornelisen et al. 2003). We estimated leaf area from scaled digitized images of fresh leaves using the software Image J (Rasband 2004). Specific leaf area was estimated by dividing the leaf fresh area by its oven-dried (80°C for 72 h) mass. Leaf nutrients were measured in a sample of approximately 100 g of leaf collected with the same criteria as above. Leaf nitrogen and phosphorus concentration were determined using colorimetry and emission spectrometry (induced argon plasma), respectively, following Jørgensen (1977). Leaf potassium concentration was determined using atomic absorption, following Zagatto et al. (1979). For each trait, we calculated mean trait values per plot to scale up traits measured at the individual plant level to the community level.

### *Soil data*

We sent composite soil samples from each plot to the Soil Science Laboratory at the University of São Paulo for chemical analyses. We determined soil organic matter content (OM) and the concentration of available phosphorus (P), total nitrogen (N), cations (Ca, Mg, K) and aluminum (Al). The chemical analyses followed the methods proposed by Raij et al. (1987), Embrapa (1997) and Silva (1999). Organic matter was determined by organic carbon oxidation with potassium dichromate and subsequent potassium dichromate titration with ammoniac ferrous sulfate, using 0.5 g of soil and 10 ml of potassium dichromate solution. A correction factor (1.33) was used to compensate partial carbon oxidation. Total nitrogen was determined by digestion with H<sub>2</sub>SO<sub>4</sub>, followed by distillation with NaOH, using from 0.5 to 1 g of soil, 1 g of H<sub>2</sub>SO<sub>4</sub> and 15 ml of NaOH. Available phosphorus was determined by spectrophotometry after anion exchange resin extraction, using 2.5 cm<sup>3</sup> of soil. The sum of bases was calculated as the sum of potassium, calcium, and magnesium, whereas aluminum saturation was calculated as a percentage in relation to the sum of bases plus Al<sup>+3</sup>, K<sup>+</sup>, Ca<sup>+2</sup>, Mg<sup>+2</sup> and Al<sup>+3</sup> were extracted with 1 M KCl, using 10 cm<sup>3</sup> of soil and 100 ml of solution. Potassium, calcium, and magnesium were then determined by an EDTA complexometry. Aluminum was determined by NaOH titration.

### *Diversity indices*

We measured both alpha and beta diversity, and we considered traditional (non-phylogenetic) as well as phylogenetic-based indices. Alpha diversity indices enable the detection of shifts along the CCI gradient in fine scale taxa co-occurrence (community level). We used species richness, Shannon's diversity index, and mean phylogenetic distance (MPD;

Webb *et al.* 2002) as measures of alpha diversity. Beta diversity measures are useful for identifying switching points in community composition at the landscape scale (Graham and Fine 2008, Beselga *et al.* 2010, Ives and Helmus 2010). Furthermore, shifts in phylobetadiversity associated with changes in abiotic conditions and plant traits are expected to give insights into the patterns of trait evolution among species (Graham and Fine 2008). Thus, we used phylogenetic community distance (PCD; Ives and Helmus 2010), Euclidean community dissimilarity (both abundance-weighted and based on species presence/absence) and Jaccard's beta-diversity (calculated as the sum of the two components, the spatial turnover, and nestedness; Beselga *et al.* 2010, Ives and Helmus 2010). We computed these beta diversity indices as cumulative values along the CCI gradient to search for critical switching points in diversity or phylodiversity associated with community closure. To calculate phylogenetic alpha and beta diversities, we constructed a phylogenetic tree as described below.

### *Phylogenetic tree*

To calculate phylogenetic alpha and beta diversities, we constructed a phylogenetic tree for all the collected species using Phylomatic software, a phylogenetic database, and a toolkit for building angiosperm phylogenetic trees (Webb *et al.* 2008). We first assembled an initial tree using the Phylomatic software, which was based on APG III (2009); we subsequently solved polytomies by consulting available recent phylogenetic information for Myrtaceae (Costa 2009), Fabaceae (Simon *et al.* 2009), Rubiaceae (Bremer 2009) and Malpighiales (Karotkova *et al.* 2009, Bell *et al.* 2010). We also dated undated nodes based on



Bell et al. (2010). The remaining undated nodes were evenly spaced using the branch length adjustment algorithm (BLADJ) available in Phylocom (Webb et al. 2008).

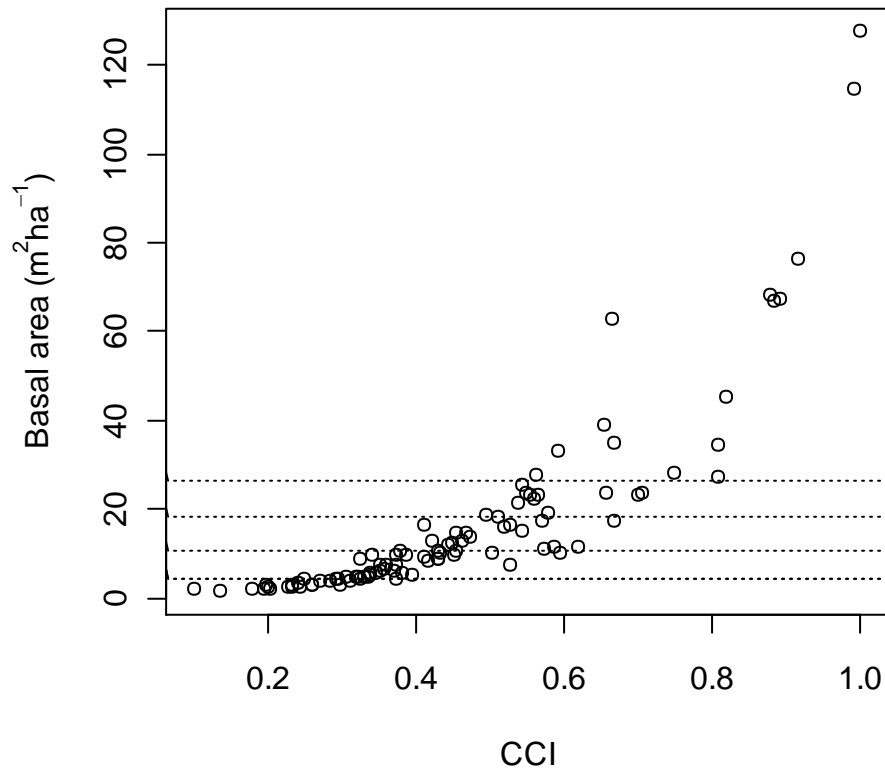
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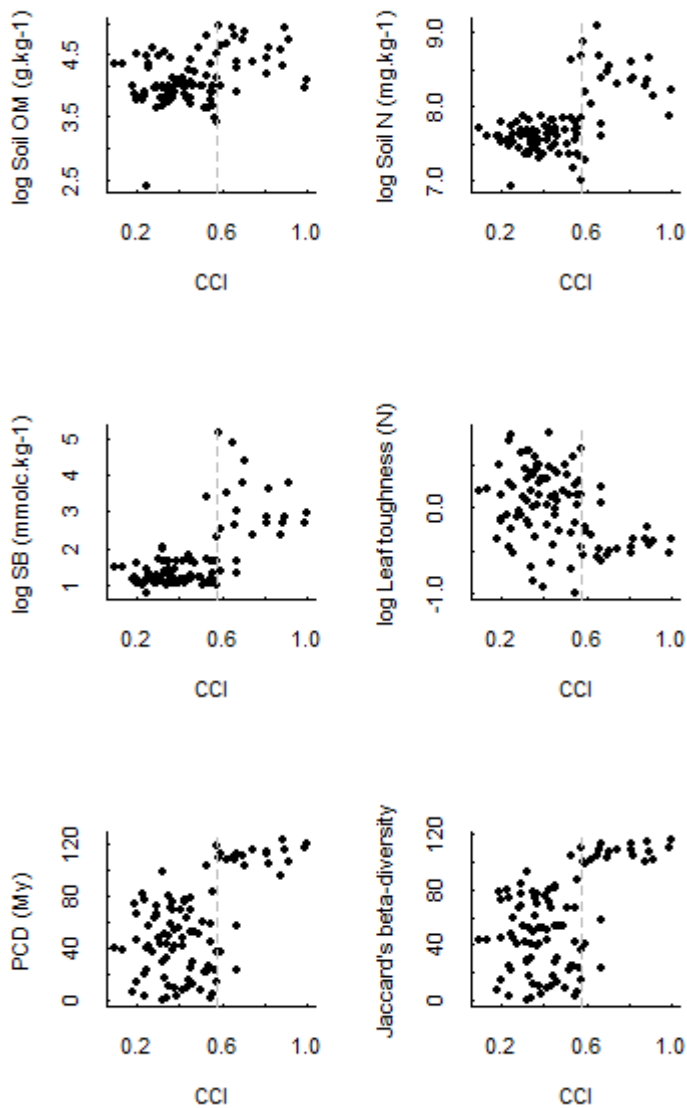
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**Appendix C: Relationship between the community closure index (CCI) and basal area per hectare.**



Horizontal lines refer to values of basal areas for the different cerrado physiognomies as studied by Hoffmann et al. (2005); from *campo sujo* and *campo cerrado* (lower line) to “cerradão” forest (upper line). The equation describing the relationship between basal area and CCI is:  $\log(\text{Basal area}) = 4.73 \cdot \text{CCI} + 0.15$  ( $R^2 = 0.90$ ;  $p < 0.001$ ). The CCI is an indicator of the light environment, from open communities (CCI close to 0) to closed communities (CCI close to 1; see the Materials and Methods section in the main text for details).

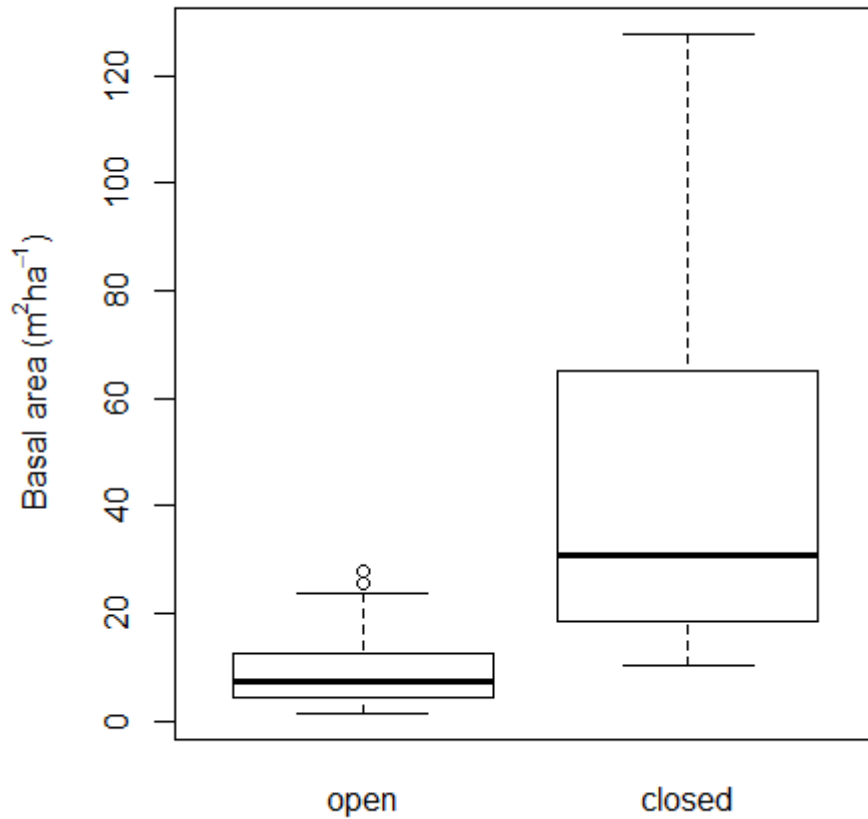
**Appendix D: Soil variables, leaf toughness and diversity indices along the community closure index gradient (CCI) and their breakpoints.**



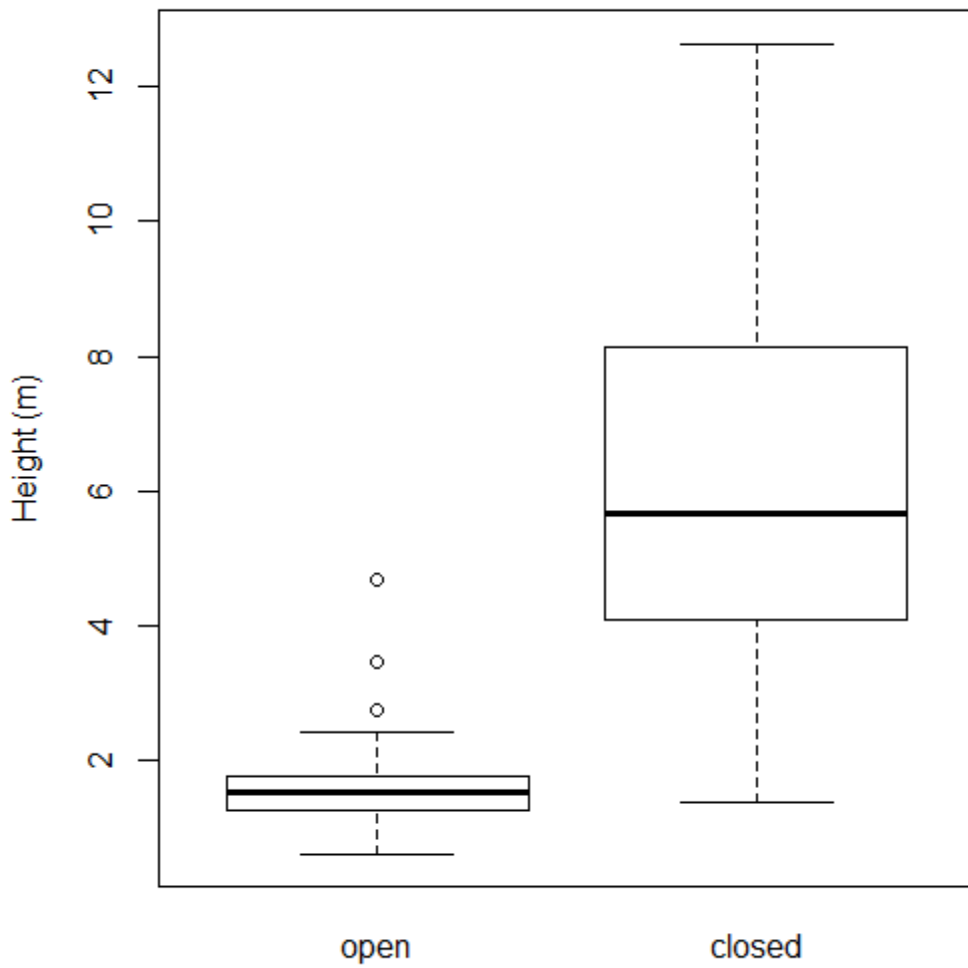
Soil organic matter (OM g kg<sup>-1</sup>), soil N (mg kg<sup>-1</sup>), soil sum of bases (mmolc kg<sup>-1</sup>), leaf toughness (N), phylogenetic community distance (PCD; My) and Jaccard's beta-diversity along the community closure index gradient (CCI, 0 to 1) as examples of the threshold-type

relationships found at Emas National Park in central Brazil. Vertical grey lines represent significant breakpoints (supF test).

## Appendix E: Distribution of basal area and mean height at each side of the threshold



Basal area (m<sup>2</sup>·ha<sup>-1</sup>) in the plots located at each side of the mean threshold along the gradient of community closure index (CCI). Open communities: CCI < 0.57; closed communities: CCI > 0.57. The CCI is an indicator of the light environment, from open communities (CCI close to 0) to closed communities (CCI close to 1; see the Materials and Methods section in the main text for details).



Mean height (m) in the plots located at each side of the mean threshold along the gradient of community closure index (CCI). Open communities:  $CCI < 0.57$ ; closed communities:  $CCI > 0.57$ .



IV – CAPÍTULO 3: A DISPONIBILIDADE DE RECURSOS MOLDA A ASSEMBLEIA  
DE SAVANAS FILTRADAS PELO FOGO\*

\* Artigo submetido ao periódico *Ecography* com o título “Resource availability shapes the assembly of fire-filtered savannas”.

## **Resource availability shapes the assembly of fire-filtered savannas**

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**Abstract** - In tropical landscapes, stabilizing feedbacks between tree cover and fire results in vegetation thresholds and create matrices of savannas that experience frequent fires spotted with forest fragments that rarely burn. The flammable savanna matrix can be considered a fire-filtered landscape because the current variation in fire frequency explains only a small fraction of the overall variability in plant traits. However, tropical savannas can be structurally and floristically patchy, and the mechanisms by which environmental drivers interact to assemble vegetation mosaics within these landscapes are not well understood. We used individual-based functional trait data, phylogenetic information and a beta community assembly framework to assess the main drivers of community assembly in a large fire-filtered savanna reserve in central Brazil. We used Mantel correlograms and a maximum rank correlation to describe the spatial structure and the subset of landscape factors that best predict the compositional, phylogenetic, species-based and individual-based functional dissimilarities among communities. All of the community dissimilarity metrics were spatially structured at landscape-to-regional scales and exhibited spatial clustering at short distances and overdispersion at greater distances. Moreover, all of the dissimilarity metrics presented unique spatial structures and were correlated with different sets of landscape predictors, exhibiting a high degree of complementarity. After accounting for the effects of the environmental predictors, most of the spatial clustering/overdispersion signals disappeared. Soil texture was the most important predictor of community assembly, but topographic features and nutrient availability were also important and interacted with evolutionarily conserved traits. As expected, the effects of fire frequency were intraspecific and resource dependent. Our results support the idea that the patchy distribution of resources, particularly those that are associated with soil texture variations, is a key factor in assembling vegetation mosaics in fire-filtered savannas landscapes.

## **Introduction**

Understanding how biotic interactions and abiotic filters synergistically drive coexistence across spatial scales is a central issue in ecology. In the last decade, information on phylogenies and functional traits has been combined with information on resource availability gradients and disturbance regimes to provide a deep understanding of the mechanisms by which species are assembled within communities (e.g., Webb et al. 2002, Verdú and Pausas 2007, Kraft et al. 2008, Pausas and Verdú 2008, Batalha et al. 2011, Cianciaruso et al. 2012). Using such information to understand the assembly of communities within landscapes (sometimes referred as beta community assembly) is currently emerging as a prominent tool for linking ecological, historical and biogeographical patterns (Hardy and Senterre 2007, Bryant 2008, Graham and Fine 2008, Swenson et al. 2011, Hardy et al. 2012, Swenson et al. 2012).

In tropical landscapes, stabilizing feedbacks between tree cover and fire drives major functional shifts maintaining two stable states: a state in which fires are uncommon and of low intensity (forest communities) and a state maintained by recurrent fires (grasslands, savannas and open woodlands communities, referred collectively as “savannas”; Hoffmann et al. 2012, Murphy and Bowman 2012, Dantas et al. 2013a). A closer look at many savanna landscapes reveals that they comprise a heterogeneous savanna matrix with frequent fires that is spotted with infrequently burned forest patches (Dantas et al. 2013a). In the flammable savanna matrix, fire acts as an environmental filter, allowing only species with fire-resistance traits to survive (Simon et al. 2009, Hoffmann et al. 2012, Dantas et al. 2013a). Thus, although often floristically and structurally patchy (Goodland and Pollard 1973, Hoffmann et

al. 2005), these communities are fire-filtered in the sense that the fire frequency variation within the ranges naturally occurring in these savannas does not explain the observed large variability in trait distribution (Dantas et al. 2013a, 2013b).

These patchy savannas are particularly well represented in the neotropics, where the most species-rich and structurally heterogeneous (on a climate basis) tropical savannas occur (Simon et al. 2009, Murphy and Bowman 2012). In these savannas, tree density can be highly variable within climatically homogenous landscapes (Goodland and Pollard 1973, Ruggiero et al. 2002, Hoffmann et al. 2005, Rossatto et al. 2012, Dantas et al. 2013a). Although general consensus exists that interactions among soil fertility, water availability and fire frequency influence these structural mosaics (Goodland and Pollard 1973, Kellman 1984, Ferreira et al. 2007, Dantas and Batalha 2011, Hoffmann et al. 2012, Rossatto et al. 2012), little is known about how they synergistic interact to drive the patterns of community assembly and at what spatial resolution and level of organisation (species or individual) they operate. Moreover, most of the studies on these savannas have investigated the role of a single predictor in explaining woody plant densities, species richness or basal areas (Goodland and Pollard 1973, Ruggiero et al. 2002, Ferreira et al. 2007, Dantas and Batalha 2011). However, approaches other than functional or phylogenetic approaches can ignore important sources of variability and miss the spatial scale at which predictors operate, possibly leading to conflicting conclusions (Goodland and Pollard 1973, Haridasan 1992, Ruggiero et al. 2002, Marimon Jr. and Haridasan 2005, Viani et al. 2011). A multivariate functional and phylogenetic approach might provide important mechanistic insights into how overlapping predictors interact with the functional attributes of individual plants to shape the assembly of these savanna communities.

The present study attempts to answer the following questions: (1) what are the main predictors shaping the spatial structure of savanna communities in fire-filtered landscapes? (2) By what mechanisms do these predictors operate to shape community assembly? (3) What are the spatial scales at which these predictors operate? To answer these questions, we studied 100 plots, 0.019 to 54 km apart, distributed across the heterogeneous landscapes of Emas National Park, a 132,000 ha cerrado reserve in central Brazil, and explored the relationships among compositional, phylogenetic, species-based and individual-based functional dissimilarities across spatial and environmental gradients. In tropical savannas, the patchy distribution of resource availability interacts with fire events of variable intensities to influence both the outcome of tree-grass competition and the fire escape probability of young individuals, exerting both recruitment and establishment bottlenecks (Kellman 1984, Bond 2008, Hoffmann et al. 2012, February et al. 2013). Thus, we postulate that by creating different opportunities for fire escape and affecting tree-grass competitive outcomes, variations in resource availability should explain the assembly of fire-filtered savannas. Consequently, we predict a patchy distribution for functional, phylogenetic and compositional dissimilarities that matches the spatial distribution of water and nutrient supplies. Moreover, because these communities are fire-filtered (mean fire intervals between 2 and 8 years; Dantas et al. 2013a), we expect variations in fire frequency within the current ranges experienced in the savannas to drive intraspecific trait shifts (Higgins et al. 2007, Dantas et al. 2013b); we also expect the effects of fire to be resource-dependent (Bond 2008, Hoffmann et al. 2012). Moreover, we expect resource-related predictors to explain the phylogenetic community composition of the cerrado because of the diverse ancestral habitats from which cerrado lineages evolved (both dry and rain forests with variable nutrient availability to plants; Simon

et al. 2009) and because resource-related traits are the most evolutionarily conserved traits among neotropical savanna woody plant lineages (Cianciaruso et al. 2012).

## **Materials and Methods**

### *Study site*

We used the same dataset as that used by Batalha et al. (2011), which was sampled in Emas National Park (ENP), a large cerrado reserve located on the Brazilian Central Plateau (17°49'-18°28'S and 52°39'-53°10'W). The climate of ENP is tropical and humid, and the area experiences a wet summer and a dry winter (Aw following Köppen 1931). Open cerrado physiognomies prevail (grasslands and savannas of varying tree densities) in the landscape, covering 68.1% of the total area, while woodlands cover 25.1% of the reserve (Ramos-Neto and Pivello 2000). Small forest fragments also occur scattered across the landscape but cover only approximately 1.2% of the park (Ramos-Neto and Pivello 2000, Dantas et al. 2013a); they were not considered in the present study. The soils are usually acid, nutrient-poor Oxisols, with variable textures from clay to sandy soil (Batalha et al. 2011). The mean fire intervals within the park range from 2 to 8 years, except in forest patches, which may go more than three decades without experiencing high intensity fires (Dantas et al. 2013a).

We arranged 100 5x5-m plots along ENP's network of firebreaks and small roads (see Batalha et al. 2011 for a map and the plot locations). The pairwise distances between the plots were between 19 m and 54 km (mean= 20.840 km; SD = 10.760 km; median = 20.126 km). The savanna landscapes of the park were classified into 10 strata according to the time since the last fire, and 10 plots were randomly set within each stratum. This sampling approach

allowed the inclusion of communities that were highly variable in vegetation structure, topography, soil features, soil texture and mean fire interval (Batalha et al. 2011).

### *Field sampling*

In the rainy season of 2009/2010, we tagged all woody individuals with stem diameter greater than or equal to 3 cm at the soil level, identified them to species, measured the functional traits and environmental predictors in all plots, and collected material for laboratory analysis (see Batalha et al. 2011). Specifically, we measured plant height, the circumference at ground level, bark thickness, leaf toughness and topographic features (altitude and slope) in the field and collected wood, leaf and soil material for laboratory analysis. The depth to which a knife penetrated five haphazardly selected points on the main stem (50 cm from the ground) was measured using a calliper to determine bark thickness. Leaf toughness was measured using a gauge penetrometer equipped with a cone tip (Chatillon DFE 010, AMETEK, Berwyn, Pa., USA) on five haphazardly selected (fully expanded and hardened) leaves per individual. Leaves were penetrated on both sides of the midrib for a total of 10 measurements per individual (5 measurements at each side of the midrib). We collected five soil subsamples (top 10 cm) in each plot, one at each corner of the plot and one at the centre; the subsamples were then combined into a single sample to estimate the soil nutrients and texture. The topographic features were expected to relate to water availability; as in most of the cerrados of central Brazil, the topography varies smoothly and is associated with groundwater depth, with seasonally waterlogged areas downslope near rivers and with deep groundwater levels upslope (Rossatto et al. 2012).



### *Laboratory analyses*

The samples and the information collected in the field were analysed in the laboratory to determine basal area, mean bark thickness, mean leaf toughness, wood density, leaf size, specific leaf area and leaf nitrogen (N), phosphorus (P) and potassium (K) concentrations for each woody individual (see Batalha et al. 2011). We determined the leaf size and specific leaf area based on five haphazardly collected expanded and hardened leaves per individual, and wood density was determined based on a single cylindrical sample of wood per individual. Leaf size was determined by scanning the leaves while fresh and calculating their areas. Specific leaf area was obtained by dividing the area of the fresh leaf by its oven-dried weight (80°C for 72 h). We submitted leaf samples to the University of São Paulo for leaf N, P and K determination.

We expected the measured traits to be related to several important axes of plant strategies in the cerrado. However, the mechanism driving shifts from species average values may differ from mechanisms generating among-species dissimilarity. Whereas functional differences among species are generally associated with different plant strategies and species-specific trade-offs, there are many possible drivers of shifts from species average values (intraspecific shifts). At the interspecific level, we expect trait variation to be related to plant nutrient (specific leaf area, leaf toughness and nutrient concentrations), water storage/transport (wood density; Bucci et al. 2004), and allometric strategies (basal area, plant height and bark thickness; Gignoux et al. 1997, Dantas and Pausas 2013) and also to trunk resistance/recovery rate trade-off (wood density; Curran et al. 2008). On the other hand, we expect trait variation at the intraspecific level to be related to the opposing phenotypic effects of plant growth

enhancers (all traits; Bucci et al. 2006, Gonçalves-Alvin et al. 2006) and fire damage due to topkill followed by basal or epicormic resprouting (all traits; Dantas et al. 2013b).

The soil samples were analysed for pH, organic matter, total N concentration, available P, exchangeable K, exchangeable calcium (Ca), exchangeable magnesium (Mg), exchangeable aluminium (Al), sum of bases, cation exchange capacity, base saturation and Al saturation, which are considered important indicators of the fertility status of cerrado soils. Moreover, the percentages of sand, silt and clay were also determined. The soil texture was expected to relate to water retention, with soils containing more sand (larger particles) retaining less water than soils containing more clay (Salisbury and Ross 1992). Moreover, we also expected soil texture to relate to nutrient availability because clay particles, which have negatively charged surfaces, attract both water and cations (Salisbury and Ross 1992). Because great differences in rainfall incidence among communities are not expected to occur, soil texture is expected to be an important indicator of the water availability to plants. For a detailed description of the soil methods used, see the Supplementary Materials in Dantas et al. (2013a). We also determined the mean interval between consecutive fires for each plot based on images from the Landsat 5 and 7 satellites (TM and ETM; 30 m resolution) for the years between 1984 and 2009, using the inverse of fire frequency, as described in Dantas et al. (2013a).

### *Functional, Phylogenetic and Compositional Dissimilarities*

We calculated four among-communities dissimilarity matrices using: (1) Sørensen's compositional dissimilarity (CDiss; Sørensen, 1948), (2) phylogenetic dissimilarity (PDiss;

Bryant et al. 2008), (3) species-based (FDiss) and (4) individual-based functional dissimilarities (iFDiss).

To calculate phylogenetic dissimilarity, we constructed a phylogenetic tree for all of the sampled species using the software program Phylomatic (Webb and Donoghue 2005). We began by estimating the phylogenetic relatedness among all of the species sampled, based on the current Phylomatic tree (R20091110; APG 2009). We improved tree resolution using recent phylogenies for Fabaceae (Simon et al. 2009), Malpighiales (Karoťkova et al. 2009, Bell et al. 2010) and Myrtaceae (Costa 2009), and dated the nodes based on Simon et al. (2009) and Bell et al. (2010). Undated nodes were placed evenly between dated nodes using the “branch length adjustment” algorithm of ‘Phylocom’ (Webb et al. 2008). We calculated PDiss as the inverse of the branch length shared by the taxa of two communities using a phylogenetic tree and a community presence-absence matrix (Bryant et al. 2008). FDiss was calculated in the same way as PDiss but using a functional dendrogram instead of a phylogenetic tree. To construct the functional dendrogram, we calculated mean trait values for each species and all 10 traits, and computed hierarchical clustering using the unweighted pair group method for the species-by-trait matrix. A similar procedure was used to calculate iFDiss, but the analyses were performed using individual data rather than species mean data. Although many studies have investigated trait variation using isolated traits, a multivariate approach was preferred because the environmental effects on particular traits are not independent from those on other traits due to evolutionary trade-offs. This approach allowed us to capture how environmental predictors shape the assembly of communities through its effects on individual plants instead of particular traits, which provides a much more realistic view of the plant phenotype that interacts with the environment (Ricklefs and Travis 1980).

Thus, this approach should provide a better assessment of the patterns of community assembly (Pausas and Verdú 2008).

### *Statistical analyses*

We first explored whether CDiss, PDiss, FDiss and iFDiss were spatially structured in the landscape using mantel correlograms (1,000 randomisations; Bocard and Legendre 2012). This approach allowed us to evaluate whether, for a given distance class, communities are more similar (spatial clustering) or more dissimilar (spatial overdispersion) than expected by chance and to assess the spatial resolution of these signals (Borcard and Legendre 2012). We then selected the best subset of landscape predictors explaining variations in CDiss, PDiss, FDiss and iFDiss using maximum rank correlation as implemented by the function ‘bioenv’ of the R package ‘vegan’ (Clarke and Ainsworth 1993, Bryant et al. 2008). This function searches for the combination of environmental predictors that maximises the Pearson’s correlation coefficients between the dissimilarity matrix and the distance matrices generated from every possible combination of environmental predictors (standardised to lie in the range from 0 to 1). The significance of the selected model was assessed using a Mantel test (999 randomisations). To determine which traits exerted the strongest influence on the FDiss and iFDiss matrices, we used the same procedure using trait distance matrices instead of environmental predictor distance matrices. For FDiss, we used a traits matrix in which plot mean values were calculated for each trait after assigning the overall species mean trait values to all individuals within the plot (interspecific trait variability only). For iFDiss, we used the residuals of the correlation of this matrix with a matrix of means calculated using the actual individual values. Moreover, in all analyses including iFDiss, we partitioned out the effects of

FDiss (intraspecific variability only). This approach allowed analysing the drivers of functional changes resulting from changes in species composition (FDiss) and shifts from species average values (intraspecific trait variability; iFDiss) separately.

To understand the mechanisms by which these predictors shape community assembly, we followed the framework summarised in Table 1, which allowed us to infer the level of organisation (species or individual) at which the predictors operate and whether they operate on evolutionarily conserved, evolutionarily labile or unmeasured evolutionarily conserved traits. For instance, the variables predicting the individual-based (iFDiss) but not the species-based (FDiss) functional dissimilarity statistics operate mainly on intraspecific trait variability. That is, they drive shifts away from the species-average phenotype rather than driving species turnover. When an environmental predictor is related to both species-based functional (FDiss) and phylogenetic (PDiss) dissimilarities, it indicates that this predictor drives changes in species composition and interacts with measured evolutionarily conserved traits (see Table 1 for more cases). Finally, we constructed partial mantel correlograms for CDiss, PDiss, FDiss and iFDiss, including the environmental predictors obtained during the model selection (maximum rank correlation above) as covariates (partial). This allowed us to assess the spatial scale at which landscape predictors operate and whether any spatial structure remained in CDiss, PDiss, FDiss and iFDiss after considering the effects of the environmental predictors. We conducted all of the analyses in R using the “betapart”, “ecodist”, “picante” and “vegan” libraries.

For the analyses using Mantel correlograms, we only considered the first two-thirds of the distance classes because at longer distances, the significance becomes less reliable due to the low number of plots available for comparison (Fortin and Dale 2005). The use of Mantel tests

should be avoided when alternative approaches that are not based on distance matrices are possible (Legendre et al. 2005; but see Tuomisto and Ruokolainen 2006). However, Mantel tests are among the most appropriate and robust methods for testing hypotheses based on dissimilarity metrics, as in functional and phylogenetic beta community assembly studies (Hardy and Senterre 2007, Bryant et al. 2008, Graham and Fine 2008, Bocard and Legendre 2012, Hardy et al. 2012, Pellissier et al. 2013).

## **Results**

We sampled 531 individuals belonging to 55 species from 26 taxonomic families. The most species-rich families were Fabaceae and Myrtaceae (nine species each), followed by Erythroxylaceae and Malpighiaceae (three species each). The remaining 22 families were represented by either one or two species each. Most of the landscape predictors were spatially structured across the landscape (Supplementary material Appendix 1, Table A1). For all dissimilarity metrics, the dissimilarity increased with distance (Fig. 1; Supplementary material Appendix 1, Table A2). Specifically, plots were more similar than expected by chance (clustering) in floristic, phylogenetic and individual-based functional compositions at shorter distances (< 5 km) and were more dissimilar than expected by chance (overdispersion) at larger distances (>15 km). For species-based functional composition, the spatial grain was larger, with plots more similar in FDiss at approximately 20 km and more dissimilar at approximately 30 km (Fig. 1; Supplementary material Appendix 1, Table A2).

All dissimilarity indicators were related to one or more environmental predictors (Table 2): CDiss was only related to clay content; PDiss was related to altitude, slope, soil N, sand and

clay contents; FDiss was related only to sand content; and iFDiss was related to pH, soil N, clay and fire recurrence. Thus, soil texture (sand and clay) was the most important factor explaining among-community dissimilarity over the landscape (Table 1). The main traits related to FDiss were basal area, plant height, wood density, leaf toughness, leaf size, leaf N and P concentrations ( $r = 0.52$ ;  $P = 0.001$ ), whereas the main traits expressed in iFDiss were basal area, wood density, and leaf N, P and K concentrations ( $r = 0.31$ ,  $P = 0.001$ ). Most of the previously detected spatial signals disappeared after the effects of landscape predictors were accounted for, showing at which spatial scale different landscape predictors or combinations drive major community thresholds (Figs. 1 and 2). However, four out of 10 spatial structures were not predicted by any environmental factors studied here, whereas one new structure emerged (clustering; Fig. 2 and Supplementary material Appendix 1, Table A3).

## **Discussion**

Savanna community structure varies spatially in a nonrandom pattern. All dissimilarity indicators were spatially structured and exhibited phenotypic clustering or overdispersion depending on the spatial scale. PDiss, FDiss and iFDiss all presented unique spatial structures at some scale, and none were related to the same group of environmental predictors. Moreover, although the spatial structure of CDiss was somewhat similar to the structure of PDiss, the latter presented more and stronger signals and was associated with more predictors than CDiss, which was only associated with clay content. This indicates that little redundancy exists among the functional and phylogenetic estimators of community dissimilarity because none of them alone captured the entire functional structure of the communities. Because most

of the spatial signals disappeared after considering model effects (six out of ten initial signals), our results indicate that the landscape predictors included in this study account for an important fraction of the functional heterogeneity of fire-filtered savannas. Overall, our results support the idea that even in landscapes filtered by fire for long periods, spatially structured environmental predictors operate at different levels of organisation, spatial scales and functional traits to shape community assembly.

Soil texture was the most important predictor of the functional community assembly because it was related to all metrics of community dissimilarity. Variation in soil texture is a key factor influencing the soil water retention capacity. In fine-grained clay soils, water is lost to gravitational forces more slowly than in coarse-grained sand soils (Salisbury and Ross 1992). Moreover, fine clay particles have negatively charged hydrophilic surfaces that attract both water and cations, thus affecting not only the water content but also the nutrient status of soils (Salisbury and Ross 1992). Thus, water and nutrient availability appear to be the critical factors shaping community assembly and driving shifts from the mean species phenotypes in tropical savannas. Because FDis was related only to sand content, our results provide valuable information regarding the mechanism by which soil texture interacts with functional traits to shape community composition. The main traits related to FDis were size-related traits (basal area and plant height) and physiological traits associated with both nutrient (leaf N and P concentration, and leaf toughness) and water storage/transport strategies (wood density). The association between these traits and sand content suggests that the community composition and structure are highly influenced by feedback processes between soil texture and the nutrient, water and growth strategies of the fire-resistant woody flora. Because wood density was the main trait associated with sand content (negatively associated; see



Supplementary material Appendix 1, Fig. A1), our results also suggest an important role for wood density in water storage and transport in savannas (Borchert 1994, Bucci et al. 2004).

Phylogenetic dissimilarities were related to topography (altitude and slope), soil acidity and N content. In the cerrados of central Brazil, variations in topography are closely related to groundwater depth, with seasonally waterlogged areas downslope close to rivers and deep aquifers upslope (Rossatto et al. 2012). Some woody species in the cerrado have deep taproots that allow them to exploit water at great depths, but many do not (Oliveira et al. 2005, Goldstein et al. 2008). Although we did not measure important traits related to the depth of water uptake, the association between phylogenetic dissimilarities and topographic features suggests that these traits are evolutionarily conserved (as is specific root length in North American trees; Comas and Eissenstat 2009). However, further studies would be necessary to confirm this hypothesis. Variations in pH are expected to affect biotic activity and macronutrient mobilisation strongly (Salisbury and Ross 1992), whereas N has been identified as an important factor influencing plant growth patterns in neotropical savanna species (Bucci et al. 2006). Together, these results provide further support for the idea that feedbacks between the nutrient and water strategies of plants and the nutrient and water status in the soil shape the assembly of fire-filtered savannas.

After soil texture, the most important landscape predictors of iFDiss were fire recurrence, N content and soil acidity, whereas the traits explaining iFDiss were basal area, wood density, and leaf N, P and K concentrations. The mechanisms driving shifts from species average values often differ from the mechanisms that generate among-species dissimilarities. Among species, functional differences are generally associated with plant strategies and trade-offs (Bucci et al. 2004, Curran et al. 2008, Dantas and Pausas 2013). In contrast, the drivers of

trait shifts from species mean values (intraspecific shifts) may be diverse and is expected to result from the opposing effects of plant growth enhancers and fire damage in savannas (Bucci et al. 2006, Dantas et al. 2013b). Thus, our results are consistent with the escape hypothesis, in which factors that enhance plant growth interact with factors that prevent it (e.g., fire events) to shape the structural features of savannas (Kellman 1984, Higgins 2000, Bond et al. 2008, Hoffmann et al. 2012). In neotropical savannas, plants are fully burned or scorched for protracted periods and staying in this ‘fire trap’ for a long time result in intraspecific shifts not only in structural but also in physiological leaf traits (Dantas et al. 2013b). However, because the vegetation structure is affected by both species composition and intraspecific shifts, these effects should not be interpreted as explaining all of the variation in the vegetation structure of these savannas.

Although most signals disappeared when the effects of landscape predictors were considered, some spatial structure remained (Fig. 2). Because there were no important geographic barriers separating these communities (i.e., the rates of dispersal are expected to largely override the rates of speciation), we suggest that these signals might be related to unmeasured environmental predictors. One signal for PDiss and another for FDiss were clustered at 20 km, suggesting that the same predictors were interacting to shape both structures and that these predictors interact with evolutionarily conserved measured traits (see Table 1). Because all of the sampled evolutionarily conserved traits are nutrient related (Cianciaruso et al. 2012), we hypothesise that nutrient availability at deeper soil layers could explain this structure. Although most of the roots of woody savanna plants are concentrated at the soil surface (Castro and Kauffman 1998), some adult savanna woody plants might have deep roots and exploit nutrients at greater depths (Oliveira et al. 2005, Goldstein et al. 2008, Bond 2010).

In tropical savanna landscapes, the functional structure of communities is primarily driven by feedbacks between tree cover and fire that create mosaics of high and low fire frequency communities (savannas and forest, respectively; Hoffmann et al. 2012, Murphy and Bowman 2012, Dantas et al. 2013a). Here, we show that within the fire-filtered savanna matrix, there are structural and functional mosaics that primarily depend on soil resource status, particularly in association with soil texture. This suggests that the availability of resources plays an important role in shaping community assembly in these savannas. Moreover, we present evidence that in these fire-filtered landscapes, the variation in fire frequency interacts with resource availability to shift attributes away from the average species values. Finally, we report the spatial scales at which landscape predictors operate to shape vegetation mosaics in savannas, an information that might be useful in setting parameters for modelling vegetation changes in savannas at continental to global scales. Overall, our results suggest that resource availability is a fundamental factor in assembling fire-filtered savanna communities at landscape-to-regional scales.

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**Table 1:** The framework used to interpret the relationships between the dissimilarity metrics and the hypothesised environmental predictors (A-F). iFDiss: individual-based functional dissimilarity, FDiss: species-based functional dissimilarity; PDiss: phylogenetic dissimilarity.

<b>Community</b>					
<b>Dissimilarity Index</b>					
<b>Environmental predictor</b>	<b>Dissimilarity Index</b>			<b>Functional changes</b>	
	<b>iFDiss</b>	<b>FDiss</b>	<b>PDiss</b>	<b>driven by</b>	<b>Traits being affected</b>
A	x			Intraspecific variability only	Phenotypically plastic traits
B		x		Changes in species composition	Species-specific evolutionary labile traits
C			x	Changes in species composition	Evolutionary conserved unmeasured traits
D	x	x		Intraspecific variability and changes in species composition	Species-specific evolutionary labile and plastic traits
E		x	x	Changes in species composition	Species specific evolutionary conserved traits
F	x		x	Intraspecific variability and changes in species	Phenotypically plastic and unmeasured evolutionary conserved

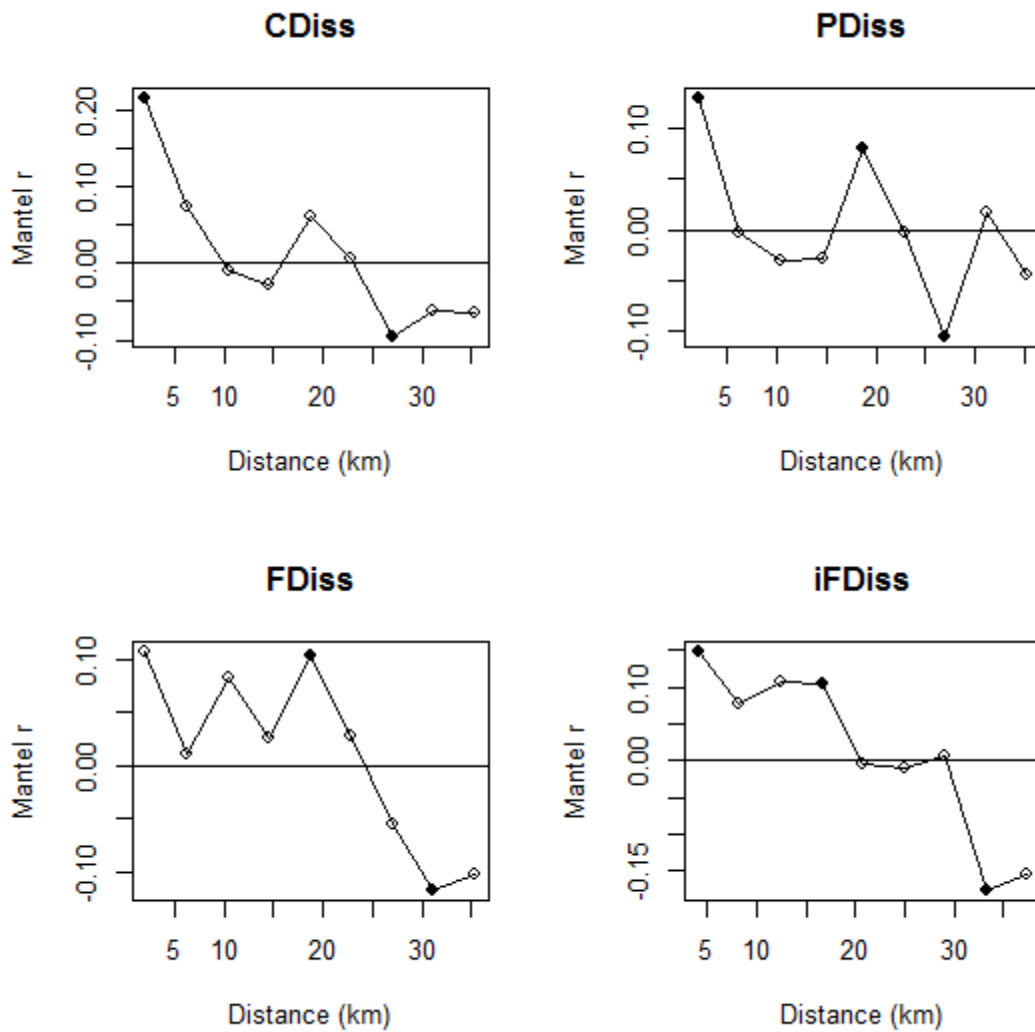
composition

traits

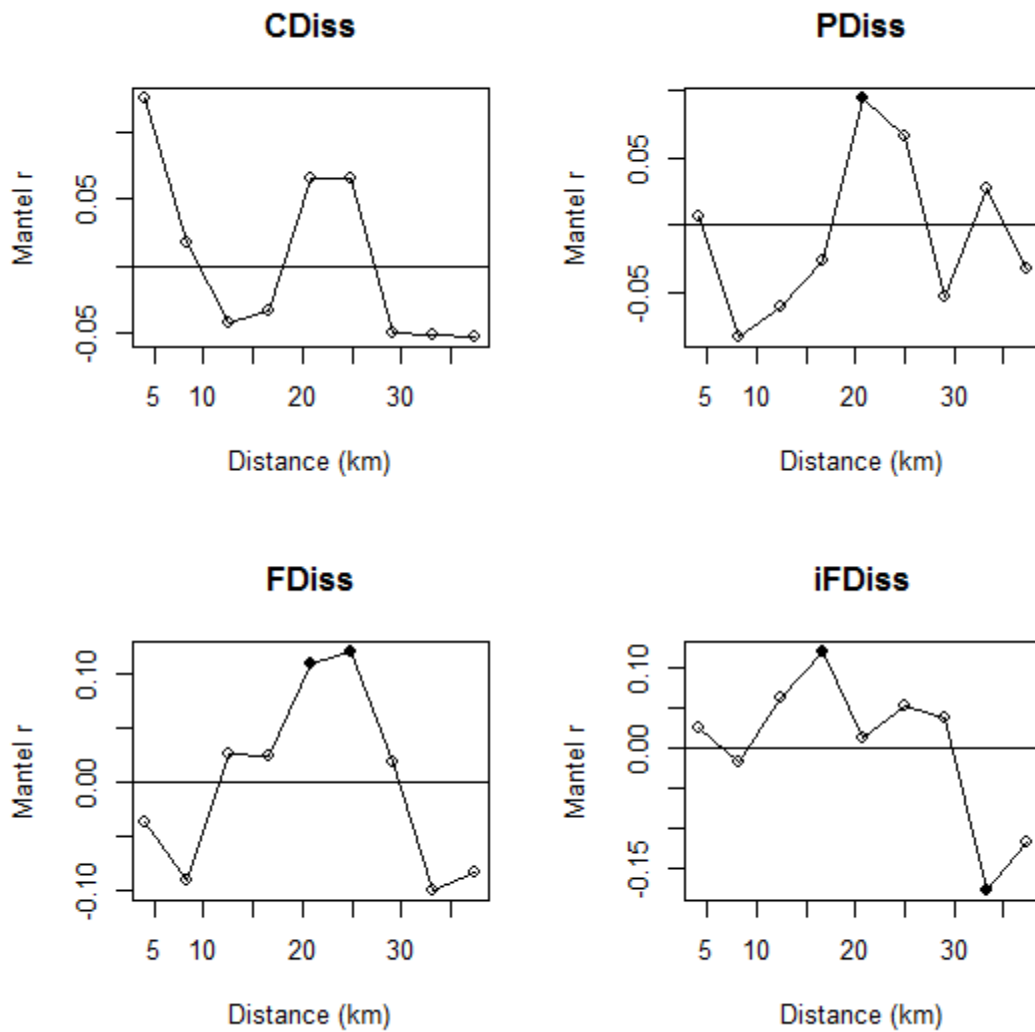
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**Table 2.** Pearson’s coefficients (*r*) for the best subset of environmental predictors of functional, phylogenetic and compositional dissimilarity, which were selected using maximum rank correlation. The *P* values were obtained by a Mantel test (999 permutations) between the dissimilarity matrix and the distance matrix from the variables in the selected models for each index (standardised from 0 to 1). CDiss: dissimilarity in species composition; PDiss: phylogenetic dissimilarity; FDiss: species-based functional dissimilarity (excluding intraspecific trait variability); iFDiss: individual-based functional dissimilarity (excluding the effects of species composition).

<b>Best model</b>		<b>r</b>	<b><i>P</i></b>
CDiss	Clay	0.15	0.001
PDiss	Altitude, Slope, N, Sand and Clay	0.19	0.001
FDiss	Sand	0.25	0.001
iFDiss	pH, N, Clay, Fire Recurrence	0.18	0.001



**Fig. 1.** The spatial structure of compositional (CDiss), phylogenetic (PDiss), species-based (FDiss) and individual-based (iFDiss) functional dissimilarities across a neotropical savanna landscape using Mantel correlograms. Significant spatial structures are indicated by black dots. Positive and negative  $r$  values indicate plots that are more similar and more dissimilar than expected by chance, respectively. For further details, see Supplementary Material, Appendix 1, Table A2.



**Fig. 2.** The remaining spatial structure of compositional (CDiss), phylogenetic (PDiss), species-based (FDiss) and individual-based (iFDiss) functional dissimilarities after the effects of environmental predictors were partial out (see Table 2 for models) using Mantel correlograms. Significant spatial structure is indicated by black dots: positive and negative  $r$  values indicate plots that are more similar and more dissimilar than expected by chance, respectively. For further details, see Supplementary Material, Appendix 1, Table A1.

## Appendix 1

**Table A1:** Results for Moran's I test of spatial autocorrelation in the predictors considered in this study. SB: sum of bases; CEC: cation exchange capacity; V: base saturation; m: aluminum saturation.

	Moran's I				
	Obs.	Exp.	Var.	ssd	P
Altitude	<b>0.635</b>	<b>-0.010</b>	<b>0.004</b>	<b>10.778</b>	<b>&lt;0.001</b>
Slope	<b>0.146</b>	<b>-0.010</b>	<b>0.003</b>	<b>2.703</b>	<b>0.003</b>
pH	<b>0.152</b>	<b>-0.010</b>	<b>0.004</b>	<b>2.734</b>	<b>0.003</b>
OM	<b>0.509</b>	<b>-0.010</b>	<b>0.004</b>	<b>8.760</b>	<b>&lt;0.001</b>
N	<b>0.239</b>	<b>-0.010</b>	<b>0.004</b>	<b>4.197</b>	<b>&lt;0.001</b>
P	0.034	-0.010	0.003	0.758	0.224
K	<b>0.178</b>	<b>-0.010</b>	<b>0.003</b>	<b>3.320</b>	<b>&lt;0.001</b>
Ca	<b>0.185</b>	<b>-0.010</b>	<b>0.002</b>	<b>4.491</b>	<b>&lt;0.001</b>
Mg	<b>0.267</b>	<b>-0.010</b>	<b>0.002</b>	<b>6.532</b>	<b>&lt;0.001</b>
Al	<b>0.170</b>	<b>-0.010</b>	<b>0.003</b>	<b>3.084</b>	<b>0.001</b>
SB	<b>0.221</b>	<b>-0.010</b>	<b>0.002</b>	<b>5.598</b>	<b>&lt;0.001</b>
CEC	-0.004	-0.010	0.002	0.135	0.446
V	<b>0.149</b>	<b>-0.010</b>	<b>0.002</b>	<b>3.784</b>	<b>&lt;0.001</b>
m	<b>0.279</b>	<b>-0.010</b>	<b>0.002</b>	<b>6.227</b>	<b>&lt;0.001</b>
Sand	<b>0.546</b>	<b>-0.010</b>	<b>0.004</b>	<b>9.349</b>	<b>&lt;0.001</b>



Silt	<b>0.422</b>	<b>-0.010</b>	<b>0.004</b>	<b>7.241</b>	<b>&lt;0.001</b>
Clay	<b>0.573</b>	<b>-0.010</b>	<b>0.004</b>	<b>9.810</b>	<b>&lt;0.001</b>
Fire	<b>0.479</b>	<b>-0.010</b>	<b>0.003</b>	<b>8.401</b>	<b>&lt;0.001</b>

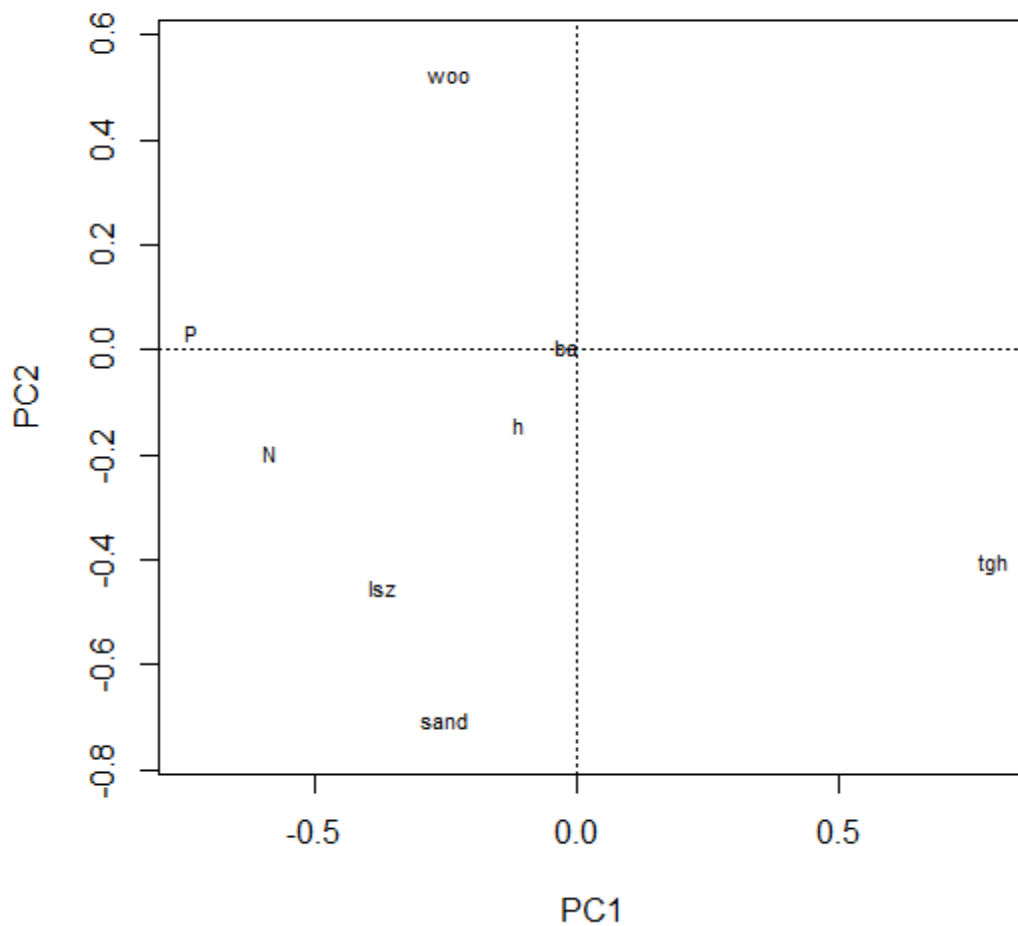
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**Table A2:** Mantel correlogram results for compositional (CDiss), phylogenetical (PDiss), species-based functional (FDiss) and individual-based (iFDiss) functional dissimilarities (lag in km). Significant spatial structures are indicated in bold: positive and negative r values indicate distances at which plots are more similar or more dissimilar than expected by chance (respectively). Persons' r and P values are presented.

n	CDiss			PDiss			FDiss			iFDiss		
	lag	R	P	lag	r	P	Lag	r	P	lag	r	P
268	<b>2.08</b>	<b>0.22</b>	<b>0.001</b>	<b>2.08</b>	<b>0.13</b>	<b>0.043</b>	2.08	0.11	0.108	<b>4.16</b>	<b>0.15</b>	<b>0.039</b>
383	6.24	0.08	0.148	6.24	0.00	0.959	6.24	0.01	0.849	8.32	0.08	0.212
505	10.40	-0.01	0.840	10.40	-0.03	0.494	10.40	0.08	0.088	12.47	0.11	0.076
674	14.55	-0.03	0.424	14.55	-0.03	0.436	14.55	0.03	0.510	<b>16.63</b>	<b>0.11</b>	<b>0.037</b>
690	18.71	0.06	0.083	<b>18.71</b>	<b>0.08</b>	<b>0.020</b>	<b>18.71</b>	<b>0.10</b>	<b>0.004</b>	20.79	0.00	0.946
656	22.87	0.01	0.855	22.87	0.00	0.953	22.87	0.03	0.481	24.95	-0.01	0.831
619	<b>27.03</b>	<b>-0.10</b>	<b>0.010</b>	<b>27.03</b>	<b>-0.11</b>	<b>0.005</b>	27.03	-0.06	0.187	29.11	0.01	0.934
380	31.19	-0.06	0.244	31.19	0.02	0.756	<b>31.19</b>	<b>-0.12</b>	<b>0.036</b>	<b>33.27</b>	<b>-0.18</b>	<b>0.028</b>
305	35.34	-0.07	0.229	35.34	-0.04	0.432	35.34	-0.10	0.086	37.42	-0.15	0.084
209	39.50	-0.05	0.478	39.50	0.07	0.337	39.50	-0.14	0.063	41.58	-0.14	0.297

**Table A3:** Mantel correlogram results for compositional (CDiss), phylogenetical (PDiss), species-based functional (FDiss) and individual-based (iFDiss) functional dissimilarities after the effects of environmental predictors are accounted for (lag in km; see models in Table 2). Significant spatial structures are indicated in bold: positive and negative r values respectively indicate distances at which plots are more similar or more dissimilar than expected by chance. Persons' r and P values are presented.

n	lag	CDiss		PDiss		FDiss		iFDiss	
		r	P	R	P	r	P	r	P
268	4.158	0.13	0.059	0.01	0.924	-0.04	0.625	0.02	0.763
383	8.316	0.02	0.726	-0.08	0.122	-0.09	0.145	-0.02	0.786
505	12.474	-0.04	0.370	-0.06	0.243	0.03	0.631	0.06	0.266
674	16.633	-0.03	0.409	-0.03	0.548	0.02	0.623	<b>0.12</b>	<b>0.018</b>
690	20.791	0.07	0.104	<b>0.09</b>	<b>0.023</b>	<b>0.11</b>	<b>0.02</b>	0.01	0.791
656	24.949	0.07	0.120	0.07	0.17	<b>0.12</b>	<b>0.027</b>	0.05	0.367
619	29.107	-0.05	0.263	-0.05	0.27	0.02	0.737	0.04	0.489
380	33.265	-0.05	0.373	0.03	0.653	-0.10	0.154	<b>-0.18</b>	<b>0.022</b>
305	37.423	-0.05	0.401	-0.03	0.659	-0.08	0.298	-0.12	0.172
209	41.582	-0.08	0.364	0.04	0.701	-0.18	0.128	-0.10	0.456



**Fig. A1.** The figure show the ordination of the main traits related to species-based functional dissimilarity (FDiss) and sand content, the only environmental predictor related to FDiss (Table 2), through a Principal Component Analysis. The first axis explained 33% of the variability in the data and was related to leaf toughness, nitrogen and phosphorus. The second explained 22% and was related to sand and woody density. ba: plant basal area; h: plant height; wo: wood density; tgh: leaf toughness; lsz: leaf size; N: leaf nitrogen concentration; P: leaf phosphorus concentration.

## V – CONCLUSÃO GERAL

## **Conclusão Geral**

Os trabalhos desenvolvidos na presente tese permitiram chegar às seguintes conclusões:

- As savanas tropicais úmidas são comunidades filtradas pelo fogo, de forma que variações nas frequências de incêndios dentro das amplitudes que atualmente ocorrem nessas savanas não explicam uma grande parte da diversidade funcional que é nelas observadas.

- O fogo nessas savanas funciona mais como um filtro externo, evitando que espécies de florestas colonizem essas ambientes, do que como um filtro interno, estruturando a assembleia de espécies em savanas.

- Nos trópicos úmidos, as savanas e florestas são dois estados vegetacionais estáveis mantidos por mecanismos de retroalimentação entre o fogo e a vegetação. As savanas são mantidas por altas frequências de fogo, ao passo que as florestas são mantidas pela inibição tanto da frequência como da intensidade dos incêndios.

- Em savanas filtradas pelo fogo, a distribuição em forma de manchas de recursos como água e nutrientes, principalmente relacionada a solos de diferentes texturas, é o principal fator regulando a assembleia da comunidade.